VOLUME LXXXIX NUMBER 1

THE

BOTANICAL GAZETTE

March 1930

COMPARATIVE ANATOMY AND MORPHOLOGY OF EMBRYOS AND SEEDLINGS OF MAIZE, OATS, AND WHEAT

GEORGE S. AVERY, JR.

(WITH SIXTY-EIGHT FIGURES)

Introduction

The structures of the embryos and the developmental anatomy of the seedlings of *Zea mays* L., *Avena sativa* L., and *Triticum vulgare* Vill. were selected for this study, since they are representative of the three morphological types of Gramineae distinguished by VAN TIEGHEM (27). Attention was paid to possible homologies of structure in embryos, in light of their later development; particularly to that part of the axis which in the seedling lies between the level at which the scutellum appears to be attached and the level of divergence of the coleoptile.

EMBRYO

Earlier literature includes many extensive and diverse studies on grass embryos. From observations on the embryos of rye and a few other Gramineae, SCHLEIDEN (21) concluded that the scutellum is the lateral limb or spreading part of the cotyledon, the coleoptile being the ligule. He was apparently the first to note that the coleoptile is a lateral structure. Although the epiblast is shown in his figures, it was seemingly regarded as of little morphological importance.

In the same year DE JUSSIEU (14) published his observations on several monocotyledons other than those studied by SCHLEIDEN, in-

cluding *Canna*. He interpreted the coleoptile as the true cotyledon, his argument being based principally on the fact that one embryonic leaf sheathes another, the coleoptile being the sheathing leaf that covers all the other embryonic leaves. He considered the scutellum to be a lateral outgrowth of the young axis which has the appearance of a cotyledon. He further considered it as playing the physiological rôle of a cotyledon. In fact, so often does it play this rôle that he considered the coleoptile imperfect and reduced to the state of a sheath.

LESTIBOUDOIS (15), studying the anatomical insertion of the first leaves of Gramineae, and those of maize in particular, considered the coleoptile to be the cotyledon. Studies on wheat, *Canna*, and other monocotyledons led him to the same conclusion. Substantially, then, he agreed with DE JUSSIEU. SCHACHT (20), studying species of *Agropyron*, agreed in part with SCHLEIDEN in considering the scutellum to be the cotyledon. However, he considered the coleoptile to be the second leaf of the plant.

Although GRIS (12) suggested no homologies in connection with the scutellum, he did describe it:

> By its great development, by its relations with the perisperm, by its connections with the embryo, by its anatomical relation during germination, it is the principal organ of absorption for the embryo, a sort of neutral medium between a structure being absorbed and an organism which becomes developed.

The first extensive observations to be made on Gramineae were those of VAN TIEGHEM (26). He examined several genera, and differed from his predecessors in that he interpreted the grass embryo in terms of its development. The cotyledon was to him made up of the scutellum and coleoptile, the former being absorptive and the latter of the nature of a bistipular sheath. He considered the epiblast of no morphological importance. Later VAN TIEGHEM (27) renounced many of his former views, and considered the scutellum as the first cotyledon, the epiblast representing the rudimentary second cotyledon. He further considered the coleoptile to be the two-bundled and sheathing third leaf, superposed to the first leaf or scutellum. The first green leaf, many-bundled, with open sheath surmounted by a blade, and superposed to the second leaf (epiblast), represented the fourth leaf of the plant.

HEGELMAIER (13), having worked with wheat and other grasses as well as other monocotyledons, agreed with VAN TIEGHEM's earlier interpretation in considering the cotyledon to be composed of the scutellum and coleoptile. BRUNS (4) regarded the scutellum as the functional cotyledon; the epiblast as a rudimentary one.

SCHLICKUM (22) regarded the scutellum and coleoptile as together representing the cotyledon. CELAKOVSKÝ (7) agreed in part with the early work of VAN TIEGHEM. He considered the scutellum as homologous with the blade of a grass leaf, the sheath which incases the bud (the coleoptile) corresponding to the closed ligule of a grass leaf; later, after growth begins, the latter appears like a free axillary double stipule. The epiblast was considered to be an appendage of the scutellum, representing the auricles at the base of each foliage leaf in certain grasses.

CANNON (6), studying the embryogeny of *Avena fatua*, treated the scutellum as the cotyledon and analogous to the haustoria of certain parasites of flowering plants. The coleoptile was interpreted as a ligule-like outgrowth arising from the base of the cotyledon, and homologous with the ligule of the grass blade. He further regarded the epiblast as a second cotyledon, although not homologous with a true cotyledon. He showed the epiblast arising as an outgrowth of the upper part of the coleorhiza.

COULTER (9) contended that, in the evolution of monocotyledons from dicotyledons, there was a suppression of one cotyledon. He cited maize and members of other monocotyledonous families as showing that the epiblast is a second cotyledon which attains only a rudimentary stage of development. The scutellum represented the one remaining functional cotyledon.

SARGANT and ARBER (18), after an extensive study of the embryos of several genera of grasses, concluded that the cotyledon consists of a sucking apex (the scutellum), and a sheath (the coleoptile), the latter being perhaps equivalent to a pair of stipules. This "double" interpretation of the coleoptile apparently was abandoned later (ARBER 1). They (18) considered the epiblast as an outgrowth of the cotyledon, of the axis, or of both, and as being of little morphological importance. Although changing this interpretation as to the importance of the epiblast (1), ARBER (2) still later returned to

the earlier interpretation of SARGANT and ARBER (18). WORSDELL (30), in observations on maize, barley, and other grasses, concluded that the scutellum is the blade of the cotyledon, corresponding to that of the foliage leaf of the grass. He considered the epiblast part of the cotyledon, representing the auricles which occur at the base of the blade of the foliage leaf of certain grasses, in agreement with CELAKOVSKÝ (7). BUGNON (5) regarded the epiblast as a cotyledonary leaf which is habitually atrophied, the first leaf of the plant. He further regarded the scutellum as representing the functional cotyledon, alternate distichous with the first vegetative leaf. The coleoptile was interpreted as the cotyledonary sheath.

PERCIVAL (16), reporting on wheat, considered the scutellum to be the cotyledon, constituting, with the epiblast and coleoptile, the first three leaves of the plant. SOUÈGES (23), studying the embryogeny of *Poa annua*, considered the scutellum to be the cotyledon, the coleoptile (in part) being formed from it. He further showed that the epiblast arises as an outgrowth of the coleorhiza. TOOLE (25), although not working mainly on the structure of the embryo, agreed substantially with SARGANT and ARBER. His observations were on maize.

SEEDLING

The opinions of earlier workers already cited concern only the structure of the embryo. Those now referred to deal with the seedling, in particular with that part of the axis which in maize lies between the scutellum underground, and the node at the surface of the ground where the coleoptile diverges from the axis. The corresponding structure in oats is equally prominent, while that in wheat is somewhat less conspicuous and will be referred to later.

Apparently the first reference to this structure was by CLOS (8). Although working chiefly on monocotyledons other than Gramineae, he described the seedling of maize as follows:

> That part of the axis between the attachment of the scutellum and the point of insertion of the coleoptile has a certain development. It presents a pith, a fibrovascular circle, and a cortical zone. It is a structure peculiar to monocotyledons, and maize in particular. The true stem seems to begin above the point of insertion of the cotyledonary sheath, where numerous bundles appear in the midst of the parenchyma.

He referred to this part of the axis only as the "neck."

VAN TIEGHEM (26) was the first to give particular attention to the structure. He regarded it as an elongated first node, however, belonging neither to the epicotyl nor to the hypocotyl. His argument was based on the fact that, in maize and similar types of grasses, there is an interval on the axis between the attachment of the coleoptile and that of the scutellum; the former being superposed directly above the latter and interpreted as the sheath of the cotyledon (scutellum). VAN TIEGHEM (27) renounced many of his former views on grass morphology, and interpreted the scutellum, epiblast, coleoptile, and first foliage leaf as the first four leaves of the plant.

DEBARY (10), reporting on maize, states that "the elongated internode between the insertion of the scutellum and the first sheathing leaf in the seedling of *Zea mays* shows an abnormal arrangement." Apparently he was the first to consider the interval in question as the first internode of the axis. BRUNS (4), studying several genera, including maize, also interpreted the structure as an internode. CELAKOVSKÝ (7), however, in complete agreement with VAN TIEGHEM'S earlier interpretation, held that the structure in question is of the nature of an elongated first node, belonging neither to the epicotyl nor to the hypocotyl. He applied the term "mesocotyl," which has since been in rather common use.

SARGANT and ARBER (18) considered the "mesocotyl" as unique. They regarded it as having resulted from a fusion of the cotyledonary stalk with the hypocotyl, and thus neither a node nor an internode. COULTER (9) was in complete agreement with BRUNS. WORSDELL (30) agreed with VAN TIEGHEM (26) as to the nature of the structure. BUGNON (5) apparently agreed with the interpretation of CELAKOVSKÝ. WEATHERWAX (29) called the structure in question an internode, but did not discuss it further. PERCIVAL (16) referred to it briefly as a "rhizome."

Materials and methods

The greater part of the maize (*Zea mays*) used in this experiment was of the Golden Glow variety, grown at the experimental farms of the University of Wisconsin. Other varieties studied were Golden Bantam and Ninety-Day Yellow Dent. In addition, Dwarf, Liguleless, and Chinese Suckering strains were observed. The material was

grown in 1925. The oats principally used were *Avena sativa*, although *A. fatua*, *A. strigosa*, *A. sterilis*, and *A. brevis* were examined. These were obtained from the United States Department of Agriculture, Bureau of Plant Industry. The wheat chiefly used was Turkey Red (*Triticum vulgare*), a hard winter wheat, grown in 1924–25. Less extensive observations were made on Durum and Einkorn, grown in 1921. The wheats came from the experimental farms of the University of Wisconsin.

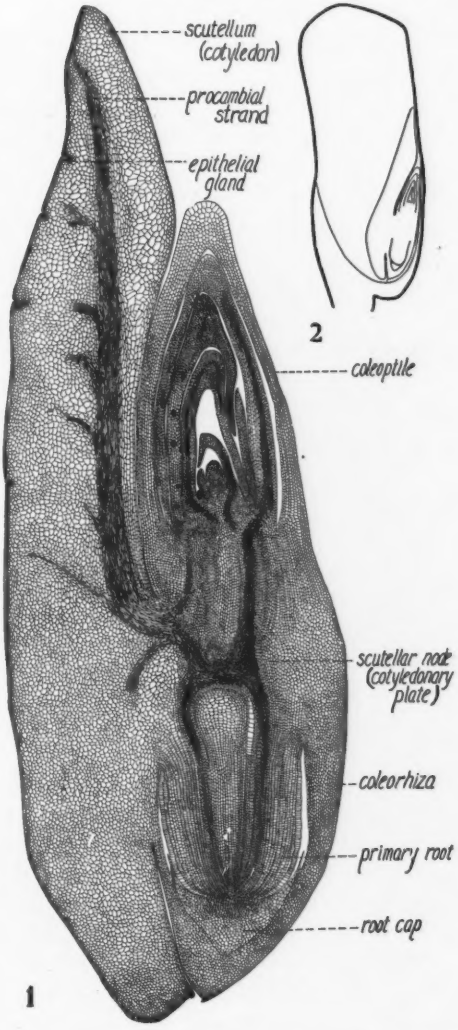
Studies were made on the material as it grew in the greenhouse and in the field. The material taken for microscopic study came from both sources, and was fixed in formal-acetic-alcohol. Many of the seedlings were dehydrated and cleared in xylol for purposes of gross observation. The material for sectioning was imbedded by the paraffin method and sectioned at a thickness of 12 μ.

In the studies on embryos, serial sections in every case were cut in transverse, longitudinal-side, and longitudinal-face sections. Conclusions were based largely on evidence gained from germinating embryos and seedlings. In addition to the embryos and seedlings of maize, oats, and wheat, those of barley, rye, and rice were observed. Although these differ structurally, they were found to agree with the interpretation reached from the study of maize, oats, and wheat.

Maize

Structure of embryo

The embryo lies imbedded in the endosperm (fig. 2), on one side and toward the base of the caryopsis. The lower end of the embryo is always nearest the narrowed end of the caryopsis. In longitudinal-side section (fig. 1) the upper part of the axis may be seen to consist of nodes bearing the primordia of leaves. The internodes are unelongated. There is a short necklike interval in the axis between the levels of divergence of the embryonic leaves and that of the scutellum. The apex of the axis consists of a small growing point. The embryonic leaves and growing point are covered by the coleoptile, making an approximately cone-shaped structure. In longitudinal-face section (fig. 10) it is possible to observe the primordia of two or more adventitious roots arising in the region just above the scutellar node. They are directed laterally upward.



FIGS. 1, 2.—*Zea mays:* fig. 1, median longitudinal-side section of embryo; fig. 2, diagram of median longitudinal-side section of caryopsis, showing location of embryo.

The scutellum is a prominent structure attached to one side of the axis. It is largely parenchymatous, and has a prominent layer of epithelial cells in contact with the endosperm tissue during germination (fig. 1). The epithelial layer often has infoldings in its surface, which have been described by SARGANT and ROBERTSON (19) as epithelial glands.

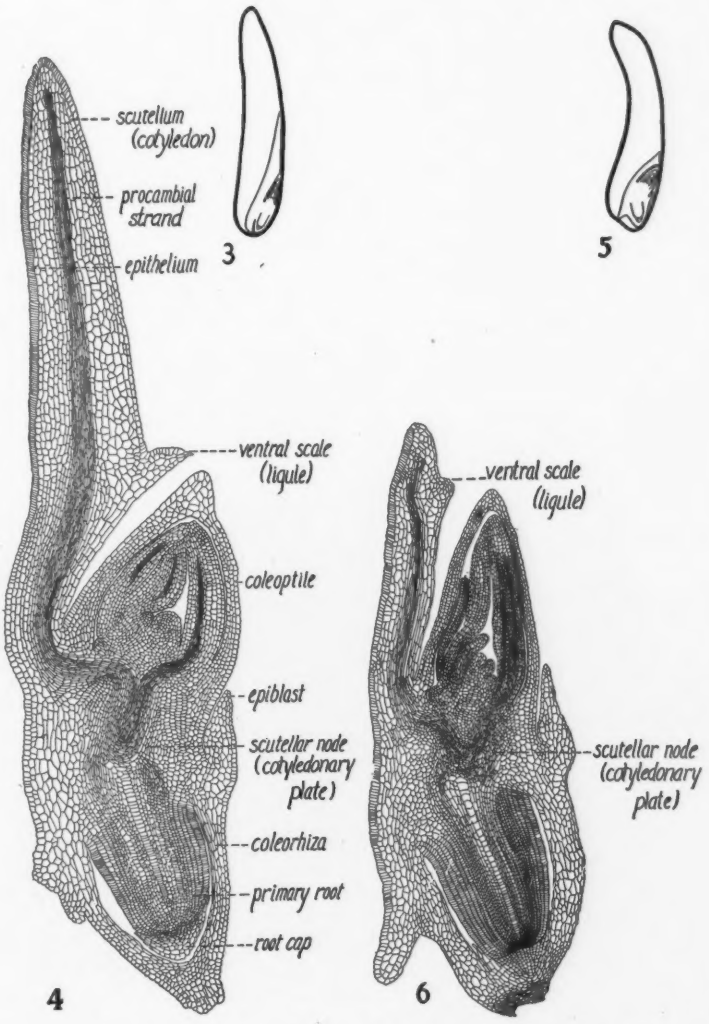
The embryonic vascular system consists partly of a large procambial strand laid down in the scutellum. This main procambial bundle branches throughout its length (figs. 1, 7). In the lower part of the scutellum are several procambial strands which radiate out and downward from the level where they diverge from the axis. In longitudinal-face section they appear much like the ribs of an inverted fan (fig. 7).

The procambial bundles from the upper and lower ends of the scutellum are joined at the level of divergence of the scutellum from the axis, and within the embryonic axis are connected directly with the stele (figs. 1, 7, 36). Continuing from the place of attachment of the scutellum bundle to the stele, it is easily possible to trace procambial elements to the first leaf above the coleoptile (fig. 1). The vascular system of the coleoptile consists usually of two vascular bundles, one on either side. They arise directly from the stele of the embryonic axis, at the upper end of the necklike interval in the axis already referred to (figs. 10, 36). They will be more fully described later.

GERMINATION

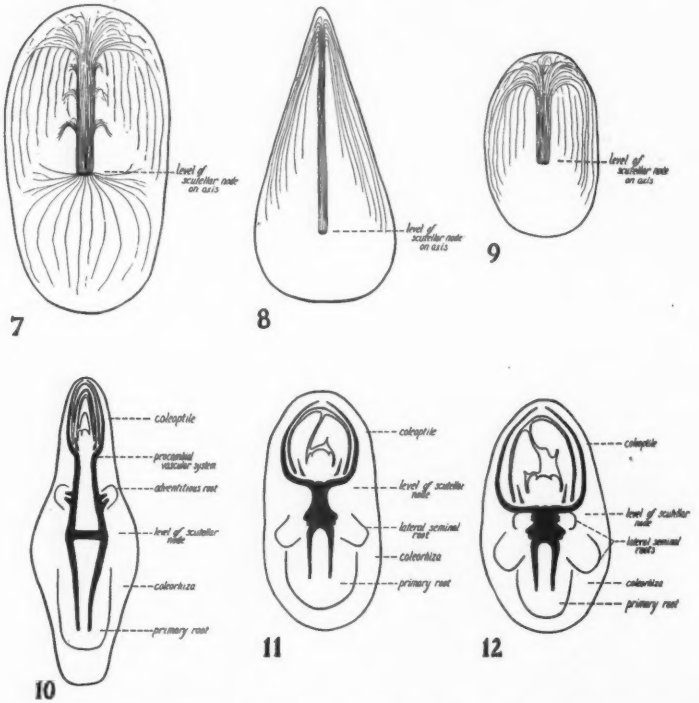
Under conditions favorable for germination, the coleorhiza bursts through the pericarp and is soon penetrated by the primary root, which grows rapidly downward (figs. 13, 14). Soon after the emergence of the coleorhiza the coleoptile emerges, the growing point of the stem together with embryonic leaves being inclosed within it. The coleoptile is pushed upward in consequence of an elongation of that part of the axis just below the level of its divergence (figs. 15, 16, 18, 19). The coleoptile remains closed until its base is level with or slightly below the surface of the soil; then its edges spread apart near its tip, on the side opposite the scutellum (fig. 16). Subsequent foliage leaves soon appear (fig. 17).

The adventitious roots, described as directed laterally upward in



FIGS. 3–6.—*Avena sativa:* fig. 3, diagram of median longitudinal-side section of caryopsis, showing location of embryo; fig. 4, median longitudinal-side section of embryo; fig. 5, *Triticum vulgare*, median longitudinal-side section of caryopsis, showing location of embryo; fig. 6, *T. vulgare*, median longitudinal-side section of embryo.

the embryo, appear early in germination (fig. 15). Although at first growing upward, they soon respond positively to the stimulus of gravity. About ten days after germination, a whorl of four or more

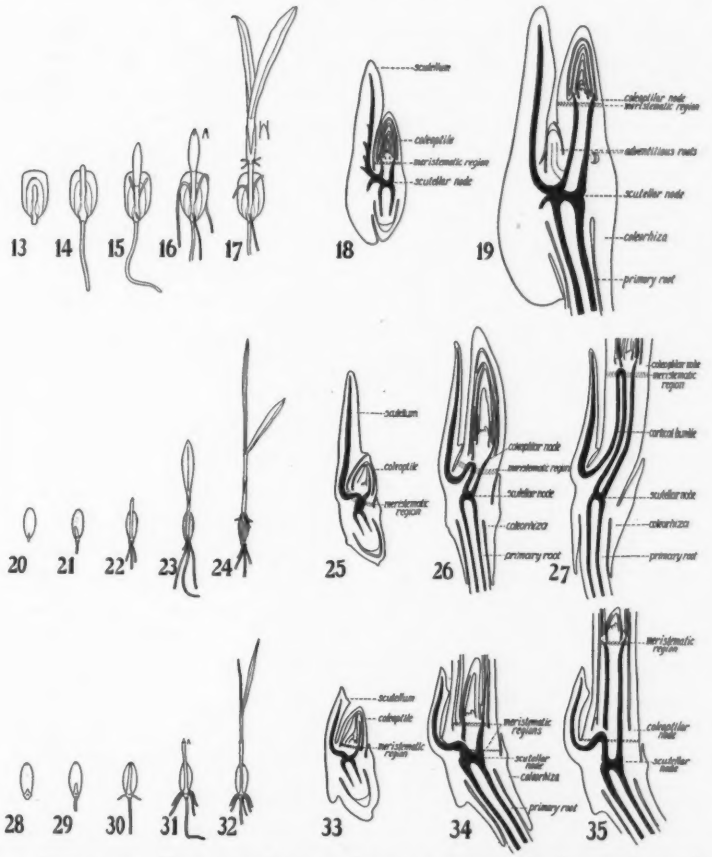


FIGS. 7–12.—Figs. 7–9, face view diagrams of scutella of *Zea mays*, *Avena sativa*, and *Triticum vulgare* respectively, showing procambial vascular systems; figs. 10–12, face view diagrams of embryonic axes of *Z. mays*, *A. sativa*, and *T. vulgare* respectively, showing procambial vascular systems in black.

roots arises from the axis just above the level of divergence of the coleoptile (fig. 17).

ANATOMY OF SEEDLING

SEMINAL ROOTS.—The primary root has a pith composed of large parenchymatous cells, isodiametric in transverse section, constituting the larger portion of the stele (fig. 57). The pith cells about the



FIGS. 13–35.—Figs. 13–19, *Zea mays:* figs. 13–17, stages of development in germination; fig. 18, median longitudinal-side section of dormant embryo (region in which first axial elongation will begin shown in dashes); fig. 19, germinating embryo, 24 hours old, showing elongation of axis between level of attachment of scutellum and that of coleoptile; figs. 20–27, *Avena sativa:* figs. 20–24, stages of development in germination; fig. 25, median longitudinal-side section of dormant embryo (region in which first axial elongation will begin shown in dashes); fig. 26, germinating embryo, 36 hours old, showing beginning of elongation; fig. 27, same, 5 days old; figs. 28–35, *Triticum vulgare:* figs. 28–32, stages of development in germination; fig. 33, median longitudinal-side section of dormant embryo (region in which principal elongation of axis will take place shown in dashes); fig. 34, same, 7 days old (slight elongation of axis just above scutellar node is starting to take place, also shown in dashes); fig. 35, same, month-old plant showing elongated second internode sheathed by coleoptile; first internode in this case shows unusual elongation.

metaxylem vessels become thick-walled within ten days after germination. The number of protoxylem points varies from eighteen to more than twice that number. There is usually one large metaxylem vessel for each two or three protoxylem groups. Alternating with the protoxylem groups are groups of phloem, each usually composed of several cells. The pericycle is characteristically a single layer of cells. Most of the cells of the endodermis have their entire inner tangential walls conspicuously thickened. The epidermis is lost early, and the walls of the outer few layers of cortical cells often become thickened. This thickening does not take place to any marked degree, however, until after the root hairs have become functionless; it is simultaneous with the thickening of the walls of certain cells of the pith, already described. Anatomically the roots arising from the axis just above the level of attachment of the scutellum are similar to the primary root. They differ in being smaller and in having fewer protoxylem points.

The structure of the first adventitious roots to appear at the surface of the ground is essentially the same as that of earlier seminal roots. They become much larger, however, and frequently have as many as forty-eight or more protoxylem points.

SEEDLING AXIS.—The first step in the "transition" from the exarch condition of the xylem of the root to the endarch condition of the xylem of the stem takes place in the vascular plate at the scutellar node (figs. 19, 42). In addition, the interval in the axis between the level of divergence of the scutellum and that of the coleoptile is a transition region. Exarch xylem groups may be observed as far up as the divergence of the coleoptile (fig. 58).

The axis above the scutellum is partly sheathed at its lower end by the scutellum (fig. 45). In transverse section (fig. 58) it resembles the primary root (fig. 57) in having small exarch xylem groups. There are, however, fewer of these groups. There is a large pith of thin-walled isodiametric parenchymatous cells. In addition, between the exarch xylem groups are endarch collateral bundles whose phloem alternates with the exarch xylem groups. The collateral bundles are not as sharply defined as are those in the upper internodes (fig. 60). The endarch and exarch bundles are arranged in a circle (transverse section in fig. 58) just inside the periphery of the

stele, similar to the circle of vascular tissue in the root. Ordinarily exarch groups and endarch bundles occur alternately, or there may be two or more endarch bundles separating successive exarch groups. The pericycle is easily identified as the prominent layer of cells immediately within the endodermis. Adventitious roots may arise from the pericycle at any level between the scutellum and the coleoptile. The radial and inner tangential walls of the endodermal cells are heavily thickened. The cortex is several cells in thickness; surrounding it is a layer of epidermal cells whose outer walls are lightly cutinized. The walls of one or more layers of cortical cells beneath the epidermis are often slightly thickened.

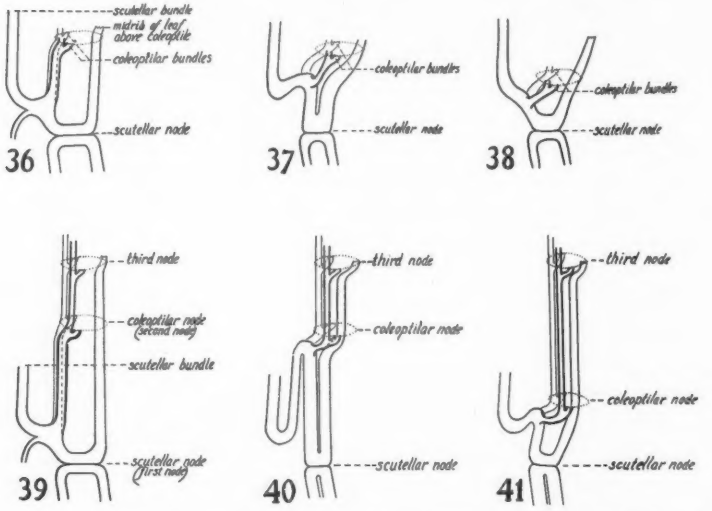
The interval on the axis between the scutellum and the coleoptile elongates by means of division and enlargement of cells just below the level of divergence of the coleoptile (figs. 18, 19). This differs from the higher internodes, which elongate as a result of activity of intercalary meristems near their base, rather than their top.

The vascular supply of the coleoptile diverges from two bundles that occur on the side of the stele toward the scutellum (figs. 36, 39). At the level of the coleoptilar node, approximately half of each of these bundles diverges laterally outward into the coleoptile. The remaining half of each bundle continues upward in the axis, giving rise to one or more traces to the leaf above the coleoptile (fig. 39). Only minor variations have been observed to take place in the origin of the vascular supply of the coleoptile and of the leaf next above it. It is obvious that the bundles of the coleoptile are ordinary leaf traces from the stele. The internode between the coleoptile and the foliage leaf next above it is sheathed by the coleoptile (fig. 48), and differs anatomically from the higher internodes (fig. 59). It possesses a rather thick cortex, very indefinite and poorly defined bundles, and an almost continuous sheath of meristematic cells among which may be found some differentiated xylem and phloem. While no particular study has been made of this meristematic sheath, it would appear to be of pericyclic origin. The internodes above the second and third foliage leaves resemble more closely the higher internodes (fig. 60).

COLEOPTILE.—The coleoptile has already been described as sheathing the growing point and embryonic leaves during the early stages of germination, and as being elevated by the growth of the

elongating axis. It has also been described as having commonly a vascular system composed of two bundles, one on either side (fig. 51). The origin of the coleoptile traces has been described.

There are exceptions to the usual number of vascular bundles in the coleoptile. DEBARY (10) notes that the two bundles usually present may each divide, making four bundles in all. This division, however, occurs before they diverge from the axis. The writer (3) has



FIGS. 36–41.[1]—Fig. 36, *Zea mays:* part of procambial vascular system of embryo, showing relation of scutellar bundle to vascular system of embryonic axis; fig. 37, *Avena sativa*, same; fig. 38, *Triticum vulgare*, same; fig. 39, *Zea mays*, showing only those bundles of seedling vascular system derived at least in part from scutellum bundle; fig. 40, *Avena sativa*, same; fig. 41, *Triticum vulgare*, same. More traces than are shown actually diverge from this source into leaf above coleoptile.

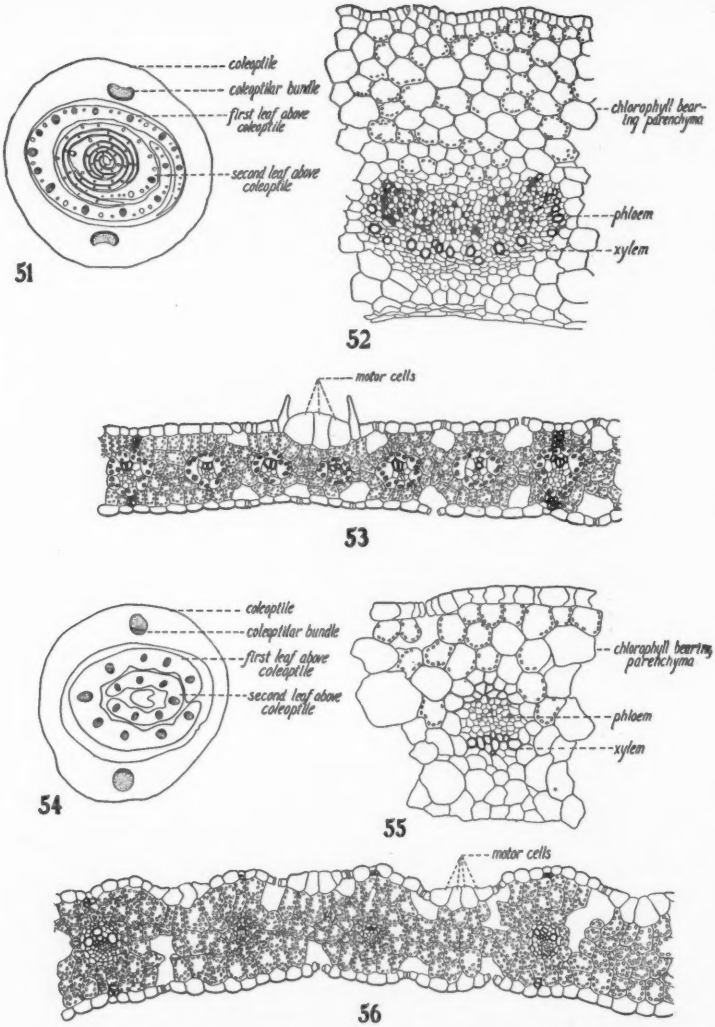
reported the presence of as many as five bundles. The extra bundles have ordinarily an origin similar to that of the usual two, although in the case of five, the fifth bundle usually arises de novo in the cortex below the coleoptilar node.

In transverse section the coleoptile displays a very simple struc-

[1] The following diagrams are designed to show destination of bundles rather than minute details of their course.

ture, being largely parenchymatous (fig. 52). The vascular bundles are large. Each bundle has many xylem groups and a considerable

FIGS. 42–50.—Figs. 42–44, diagrams of transverse sections through axes of *Zea mays*, *Avena sativa*, and *Triticum vulgare* respectively, at level of scutellar (cotyledonary) node; figs. 45–47, same, through first internode, at level immediately above first node; figs. 48–50, same, of second internode, at level just above attachment of coleoptile.

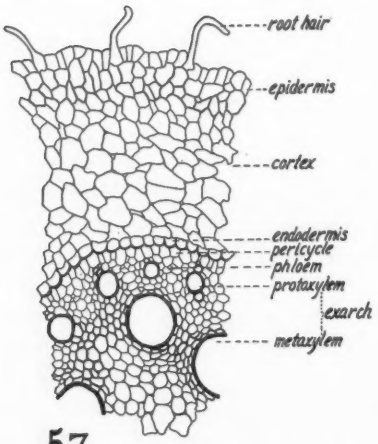


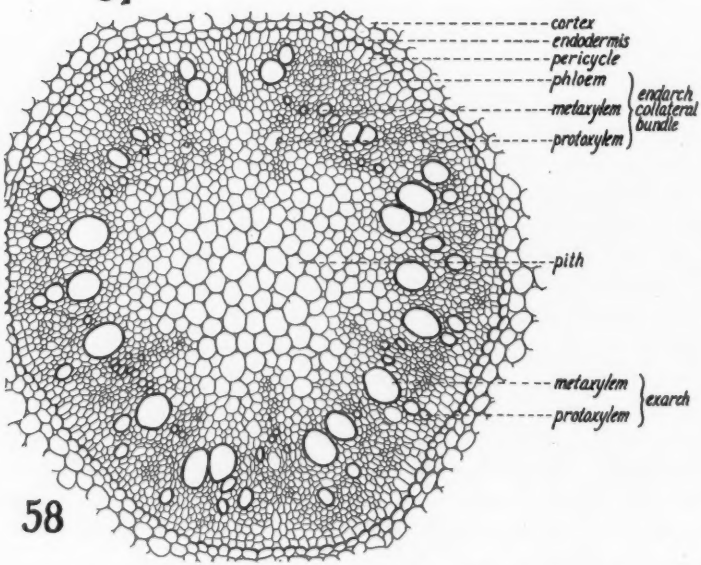
FIGS. 51–56.—Figs. 51–53, *Zea mays:* fig. 51, transverse section of coleoptile showing sheathing of later leaves; fig. 52, transverse section of small portion of coleoptile, including vascular bundle and surrounding tissue; fig. 53, transverse section of portion of later foliage leaf; figs. 54–56, *Triticum vulgare:* fig. 54, diagram of transverse section of coleoptile showing sheathing of later leaves; fig. 55, transverse section of small portion of coleoptile, including vascular bundle and surrounding tissue; fig. 56, transverse section of small portion of later foliage leaf.

amount of phloem tissue. The parenchyma making up the body of the coleoptile is closely arranged, having only small intercellular spaces. Stomata are present only in the outer epidermis. They are usually confined to one or a few rows on either side of the two vascular bundles. The guard cells are small, as are the air spaces beneath them. Chloroplasts are most numerous in the region of the bundles.

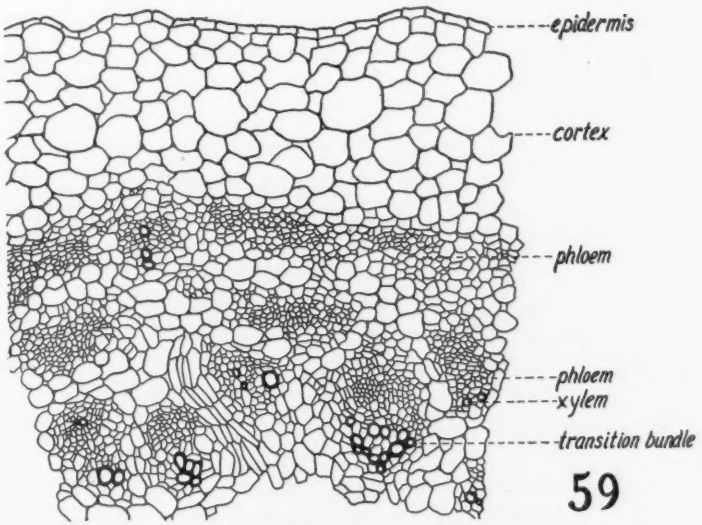
A bud was present in the axil of the coleoptile in less than 1 per cent of the maize seedlings examined (AVERY 3). Its presence appears to depend largely upon moisture and temperature conditions at the time of germination.

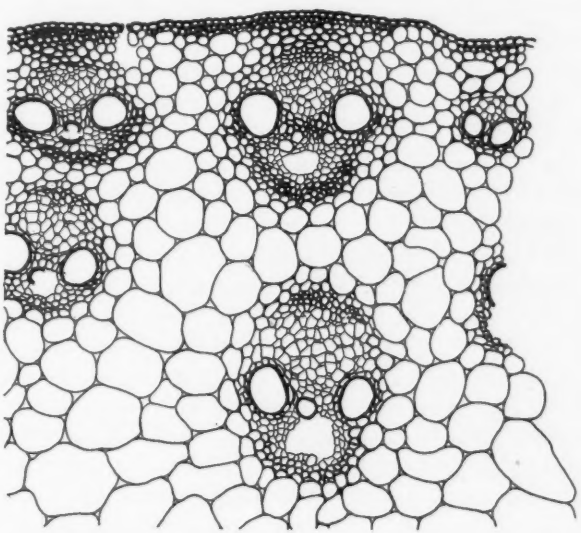
LATER FOLIAGE LEAVES.—Structurally the later foliage leaves of maize are typically similar to an ordinary grass leaf, being composed of sheath and blade. The collar-like ligule is present in most strains of maize. There are, however, liguleless strains. The blade is typically parallel-veined, but smaller cross-connecting bundles are found between the larger veins. The leaf has large and small bundles (fig. 53), the large bundles occurring between groups of from two to eight small bundles, and differing from the latter both in size and in development of mechanical tissue above and below each bundle. The mechanical tissue associated with the midrib and the larger bundles is the chief support of the leaf. Transverse sections of the sheath and blade show only minor differences. The mesophyll consists of closely arranged cells, the spongy condition being uncommon (fig. 53). There is no well developed palisade layer. The vascular bundles are surrounded by a chlorophyll-bearing bundle sheath. The chloroplasts of the bundle sheath cells differ from those of other cells of the leaf (fig. 53). The epidermis, both upper and lower, is a prominent layer of cells, the lower epidermis being more heavily cutinized than the upper. In both epidermal layers the cells are nearly square in transverse section of the leaf, but in longitudinal section are much elongated. The upper epidermis is also characterized by the presence of conspicuous hygroscopic motor, or bulliform cells, which under dry conditions apparently constitute the mechanism that functions in the rolling of the leaf. These motor cells occur in rows, and have little or no cuticle. In addition, in most varieties of maize, there are hairs on either side of these longitudinal rows of motor cells. Stomata are present in both upper and lower epidermis, and





FIGS. 57, 58.—*Zea mays:* fig. 57, transverse section of small portion of primary root of seedling; fig. 58, transverse section of stele in first internode of seedling.





FIGS. 59, 60.—*Zea mays:* fig. 59, transverse section of portion of second internode of 3-weeks-old plant; fig. 60, transverse section of portion of sixth internode of mature plant.

are arranged in rows parallel to the long axis. Epidermal cells alternate in rows with them, and under each stoma is a large intercellular space.

Oats

Structure of embryo

The embryo lies imbedded in the endosperm slightly to one side of the base of the caryopsis (fig. 3). It is opposite the groove (crease). In general appearance it is similar to the embryo of maize, but much smaller (fig. 4). The scutellum much exceeds the embryonic axis in length, is slender, and has a sharply convex face toward the endosperm. As compared with the maize embryo, it differs in at least six respects: (1) There is an epiblast (fig. 4). (2) The primordia of the seminal roots other than the primary root are usually two in number. In face section they appear in, or slightly below the region of the scutellar node (fig. 11), being directed laterally downward. A third seminal root is often present opposite the scutellum, at about the same level as the lateral roots. (3) The large procambial bundle in the scutellum shows small branch strands diverging from the upper part of the main trunk, so that the scutellar vascular system has a pattern much like the ribs of a fan, with the tips bent over toward the handle (fig. 8). There is no vascular development in the lower part of the scutellum. (4) In the dormant embryo there is no elongated interval in the axis between the scutellum and the coleoptile. (5) The epithelium of the scutellum has not been observed to possess infoldings in its surface (fig. 4). (6) On the ventral face of the scutellum is the "ventral scale," a small protrusion of tissue a considerable distance down from the apex and slightly overhanging the coleoptile (fig. 4).

Germination

Under usual conditions, structurally the external features of the germinating oat correspond with those of maize (figs. 20–24), except that there are but two adventitious roots in the region of the coleoptilar node. These are initiated just below the level of divergence of the coleoptile rather than above it, as in maize. A third may appear later, at a level just above the divergence of the coleoptile. Part of the anatomical changes during germination may be observed in figs. 25–27.

Anatomy of seedling

Seminal roots.—The primary root of the oat (fig. 61) is essentially like that of wheat (fig. 65). Structurally each primary root is characterized by a large central metaxylem vessel, which shows a lignified wall when a few days old. One or more smaller vessels may occur in the central region around the larger one, and these in turn are surrounded by parenchyma. The number of protoxylem points of the polyarch xylem varies, seven and eight being the most common numbers. Alternating with these groups are phloem areas of one to several cells each. The phloem is not well differentiated in the young seminal roots. The pericycle consists of a single layer of cells. The endodermis is characterized by thickened inner tangential walls. In the region of absorption, however, the walls of the endodermal cells opposite the xylem points are unthickened. The width of the cortex is about the same as the diameter of the stele. The epidermal cells are lightly cutinized on their outer tangential walls. As in maize, the epidermal layer is soon lost, and the layer of cortical cells next to it becomes conspicuously thickened. Anatomically the other seminal roots and the adventitious roots arising above the coleoptile are similar to the primary root.

Seedling axis.—The first step in the "transition" from the exarch to the endarch xylem condition takes place largely in the vascular plate at the scutellar node. The change is abrupt. Vascular differentiation at this level takes place as follows: When differentiation begins in the axis (during embryogeny), it takes place both upward and downward from the vascular plate. The upward differentiation results in a stele that is stemlike in part (figs. 46, 62), while downward differentiation results in typical root structure (fig. 61). Many of the xylem and phloem strands are continuous from root to stem. Above this abrupt transition region is a transition interval which extends from the level of divergence of the scutellum to that of the coleoptile. The elongation of this interval with a diagrammatic view of internal development may be observed in figs. 25–27. The location of the meristematic region in this interval is similar to that in maize. However, when elongation takes place it is seen to be slightly different, being above the vascular plate at the scutellar node and surrounding the scutellar bundle (fig. 25). Therefore the

scutellar bundle in the seedling axis extends upward parallel to, but does not become part of, the stele until it reaches the level of divergence of the coleoptile (fig. 27).

At the level of divergence of the coleoptile, the scutellar bundle forms part of the stele and turns downward within it (fig. 27). At this point two branches diverge from it which extend first laterally and then upward in the axis (figs. 37, 40). As they extend upward each branch in turn diverges. These last two traces thus derived, one on either side, are coleoptilar bundles (fig. 40). SARGANT and ARBER (18) describe the same situation by saying that half of each coleoptile bundle arises from the stele. Even though this is apparently true, the half coming from the stele comes from the same scutellar bundle that has turned downward within it. Longitudinal-face sections of the axis at this level make this unmistakable. The bundles from which the coleoptile traces are diverged extend upward, and from each, one or two (more often two) bundles diverge into the leaf above the coleoptile (fig. 40), and two or more into the second leaf above the coleoptile, etc. It should be pointed out that the scutellar bundle which turned downward in the stele at the level of the coleoptilar node, turns upward again at the scutellar node (figs. 27, 40). At this level a few vascular strands from the root add to it, and it extends upward in the stele, finally diverging as the midrib of the leaf above the coleoptile. The scutellar bundle, therefore, is entirely responsible for two (more often four) bundles of the leaf above the coleoptile, in addition to being partly responsible for its midrib. In a similar manner some of the traces of the second leaf above the coleoptile may be traced in their origin back to the scutellar bundle. Likewise the third leaf, etc.

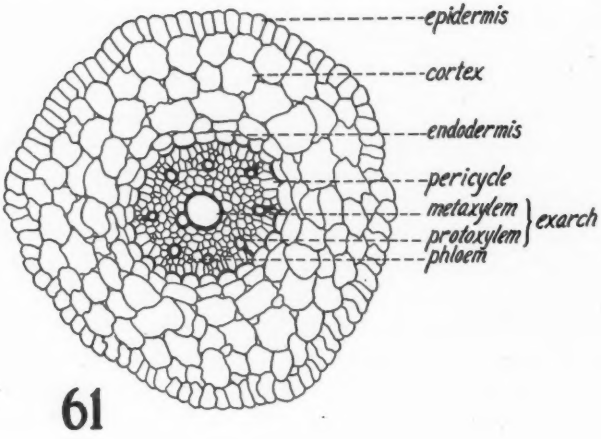
In transverse section, the stele between the level of divergence of the scutellum and that of the coleoptile is characterized by two prominent endarch collateral bundles (figs. 46, 62). They are opposite, the protoxylem of each being toward the center of the stele. In origin they are one and the same bundle, and may be traced back to the scutellum (fig. 27). The central portion of the stele is often characterized by a large intercellular space. On either side of each collateral bundle are three or four groups of protoxylem and meta-

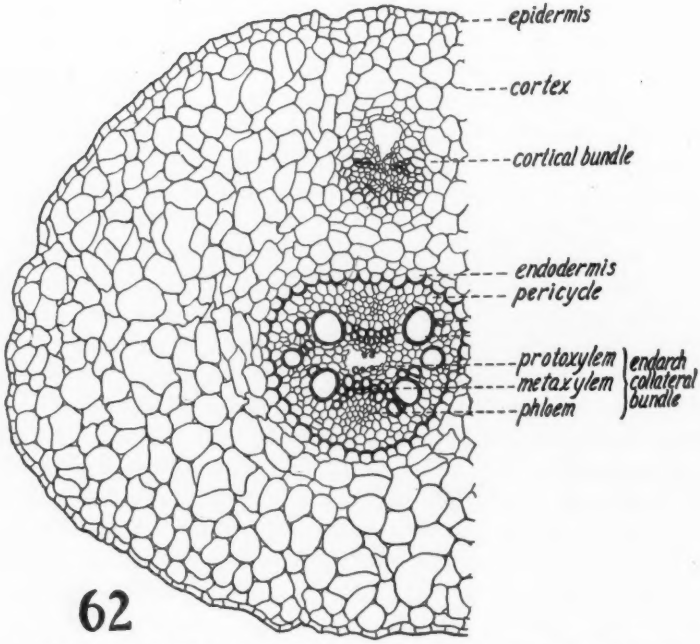
xylem vessels, some of which show varying degrees of exarch and endarch development. In the cortex just outside the stele and on the side of the axis toward the scutellum is the scutellar bundle previously described.

The internode between the coleoptile and the next foliage leaf is sheathed by the coleoptile (fig. 49). It differs anatomically from the higher internodes (fig. 63). It too is a "transition" interval, being more like the higher internodes than the internode next below it. It has a rather wide cortex, poorly organized vascular bundles, and an abundance of procambium with strands here and there differentiated into xylem and phloem. In structure the internode above the next leaf resembles more closely the higher internodes, although its bundles do not all have distinctly endarch xylem development (fig. 64).

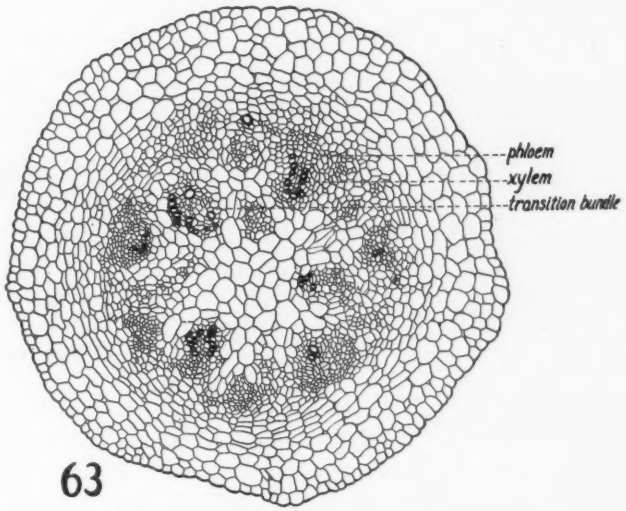
COLEOPTILE.—The coleoptile is structurally similar to that of maize, although much smaller, being about the size of that of wheat (figs. 54, 55). Like the coleoptile of maize, it too is elevated by the growth of the elongating axis beneath it. The origin of its vascular system has already been described. The coleoptile of the oat differs from that of maize in the following respects: (1) the vascular bundles are small, with few xylem groups and relatively abundant phloem; (2) a bud is almost universally present in the axil of the coleoptile; (3) no more than the usual two vascular bundles have been observed in the coleoptile.

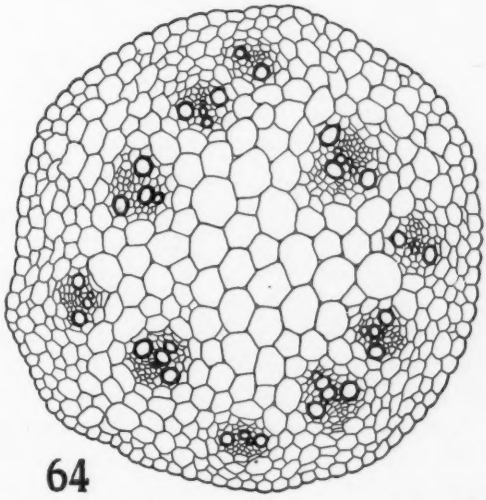
LATER FOLIAGE LEAVES.—Structurally the later foliage leaves of oat resemble more closely those of wheat (fig. 56) than of maize. Transverse sections show that the mesophyll is of much the same nature as in maize, the cells being closely arranged and with no well developed palisade layer. All the vascular bundles, except the midrib, are of essentially the same size. The mechanical tissue associated with the midrib is the chief support of the leaf. The bundle sheath cells are smaller than those of maize and do not possess chloroplasts. Usually their inner tangential walls are thickened. The motor cells are considerably less conspicuous. Frequently in both epidermal layers opposite the vascular bundles there are small, thick-walled sclerenchymatous cells similar to those found in the wheat leaf (fig. 56).





FIGS. 61, 62.—*Avena sativa:* fig. 61, transverse section of primary root of seedling; fig. 62, transverse section of first internode of seedling.





FIGS. 63, 64.—*Avena sativa:* fig. 63, transverse section of second internode of 4-weeks-old plant; fig. 64, transverse section of third internode of 6-weeks-old plant.

Wheat

Structure of embryo

The orientation of the wheat embryo in relation to the caryopsis agrees with that of the oat embryo (fig. 5). In general appearance, likewise, the two embryos are similar (fig. 6). They both possess epiblasts, similar scutellar (fig. 9), and axial vascular systems (fig. 12); and in general a similar structural makeup. Some points of difference are: (1) The primordia of the seminal roots, excluding the primary root, occur in two pairs. They appear in the region of the scutellar node, being directed laterally downward. They may be seen in longitudinal-face section (fig. 12). (2) The scutellum is a short structure, extending only slightly beyond the tip of the coleoptile. Its ventral scale is therefore near its apex (fig. 6).

Germination

In general, the early stages of germination of the wheat embryo are comparable with those previously described in maize and oats. The coleorhiza first protrudes on germination after the rupture of the pericarp of the caryopsis. It is soon followed by the coleoptile (fig. 28). The root tip then digests and forces its way through the coleorhiza (fig. 29). Its appearance is closely followed by that of a pair of lateral roots, and a second pair appears in turn just above the first (figs. 30, 31). Inasmuch as the primordia of these lateral roots were present in the dormant embryo, they may be classed as seminal roots. In longitudinal-face section (fig. 12) the origin of these lateral roots may be traced to the region of the vascular plate of the scutellar node.

At about the time that the first pair of lateral roots emerges, the coleoptile begins to elongate and the leaf primordia within begin to swell. The coleoptile serves in early germination as a protection to the primordial leaves and stem as they push their way to the surface of the ground, always elongating just ahead of them. Slightly downward from the apex, on the side away from the scutellum, there is a minute opening in the coleoptile. The coleoptile, when traced in embryological development, is found to arise as a meristematic ring of cells on the lateral face of the embryonic mass of tissue which later differentiates into the scutellum. This ring of meristematic cells de-

velops into the characteristic conelike coleoptile. As the tip of the cone gradually becomes closed, the small pore just mentioned is left. The situation is essentially similar in maize and oats. During germination, as the tip of the coleoptile reaches the surface of the ground or shortly thereafter, the foliage leaf above the coleoptile pushes through this minute pore (fig. 31). The coleoptile may or may not come above the ground before its growth ceases, depending on the depth of planting and on environmental conditions. It may contain a trace of chlorophyll for a short period after its exposure to light, and stomata appear in its epidermis both above and below ground. Subsequent foliage leaves appear in alternate distichous arrangement, and the plant remains in this condition (with unelongated internodes) for some time (fig. 32).

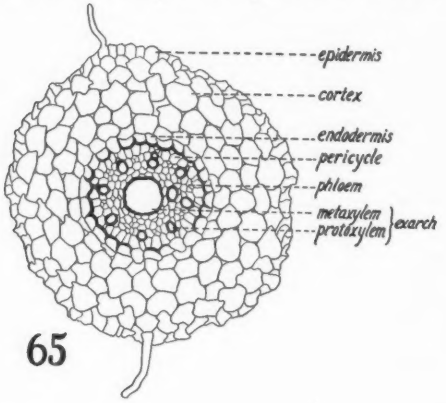
Until about two weeks after germination, no elongation of the internodes takes place except a slight elongation of the axis between the level of divergence of the scutellum and that of the coleoptile. After this period, the axis above the level of divergence of the coleoptile begins to elongate, elevating the nodes above it to approximately the surface of the ground (figs. 33–35). Throughout this time the elongating axis is sheathed by the coleoptile. A bud in the axil of the coleoptile now develops. Adventitious roots occasionally appear on the axis in the interval between the divergence of the scutellum and that of the coleoptile, and very often even above this latter level. PERCIVAL (16) contends that the adventitious roots appear in definite positions with respect to the leaves. He does not mention that roots occasionally appear at variable points in the interval between the level of divergence of the scutellum and that of the coleoptile.

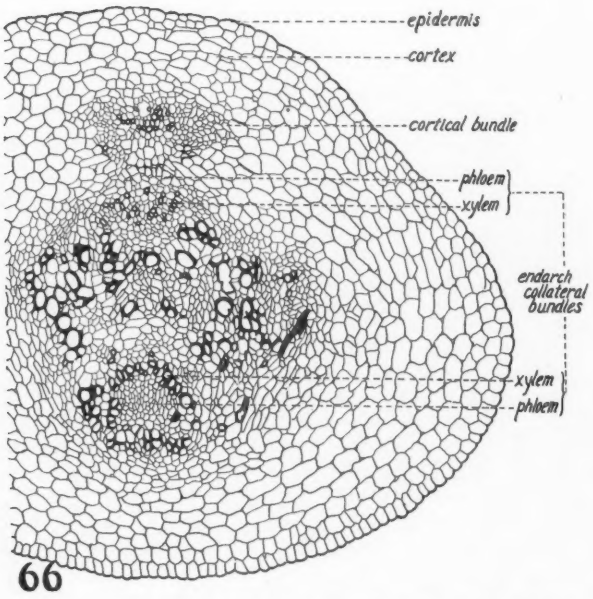
ANATOMY OF SEEDLING

SEMINAL ROOTS.—The similarity in structure of the seminal roots of wheat and oats has been pointed out. A transverse section of the primary root of wheat may be seen in fig. 65.

The adventitious roots appearing just above the level of divergence of the coleoptile are structurally similar to the seminal roots. They show several metaxylem vessels, however, instead of a large central one. The presence of more metaxylem vessels is correlated with that of a lesser amount of stelar parenchyma. This parenchyma

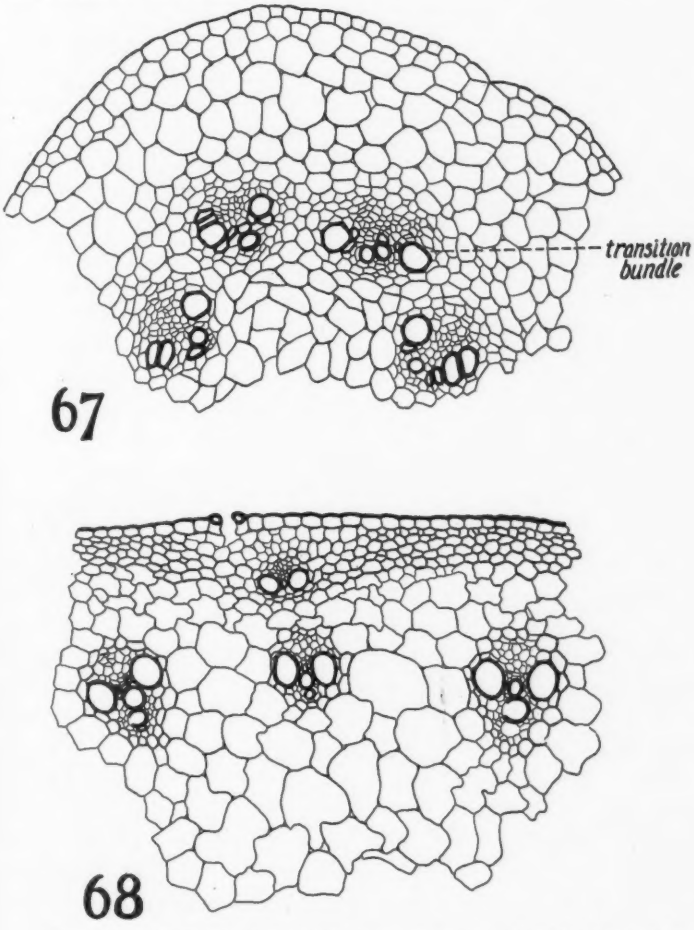
becomes lignified relatively early in adventitious roots, the protoxylem points thus becoming indistinguishable. Walls of the pericycle





FIGS. 65, 66.—*Triticum vulgare:* fig. 65, transverse section of primary root of seedling; fig. 66, transverse section of lower part of first internode of 4-weeks-old plant.

cells also become thickened. The number of protoxylem points varies from seven to eleven.



FIGS. 67, 68.—*Triticum vulgare:* fig. 67, transverse section of portion of second internode of 6-weeks-old plant; fig. 68, transverse section of portion of sixth internode of mature plant.

SEEDLING AXIS.—The first step in the "transition" from the exarch xylem arrangement in the root to the endarch condition char-

acteristic of the stem takes place in the region of the vascular plate at the level of divergence of the scutellum. Four seminal roots are initiated in this region, two on each side (fig. 12).

That part of the axis between the level of divergence of the scutellum and that of the coleoptile is very short. As previously pointed out, a slight elongation takes place in this region as the seedling develops (figs. 33–35). (The principal region of elongation in the young wheat axis is the internode above the coleoptile, as shown in fig. 35.) In the more pronounced cases of elongation, the vascular anatomy of the interval between scutellum and coleoptile is somewhat similar to the corresponding interval in the oat (fig. 62). In either case the stele in this region is largely a transitional structure. In the pronounced cases of elongation it shows in transverse section two collateral bundles. They are recognizable on opposite sides of the stele (figs. 47, 66). The arrangement of xylem, phloem, and parenchyma around them is very indefinite. Some xylem groups are exarch, some endarch, and others are indefinite. There is also a distinct bundle just outside the stele (fig. 66). With respect to this bundle the corresponding intervals on the wheat and oat axes are similar. The pericycle and endodermis are not recognizable with certainty. That part of the vascular skeleton which is derived at least in part from the scutellum, is markedly similar to that of the oat (figs. 38, 41).

The scutellar bundle within the axis diverges (frequently extending upward in the axis for a short distance before becoming part of the stele, as shown in fig. 35), the central third of it becoming part of the stele directly (fig. 41). Each outside divergence extends laterally upward for a short distance, then again diverges into two bundles, the outer one of each pair being a coleoptile trace. The inner bundle of each pair extends upward in the axis and finally diverges, giving rise to one or more bundles of the leaf above the coleoptile, as in the oat (fig. 40). The central third of the original scutellar bundle may be traced downward in the stele to the vascular plate at the scutellar node, where it turns upward. At this level a few vascular strands from the root add to it, and it extends upward in the stele, finally diverging as the midrib of the leaf above the coleoptile. The scutellar bundle is, therefore, entirely responsible for at least two bundles of the leaf above the coleoptile and partly re-

sponsible for its midrib. This is similar to the situation in oats. The vascular system of the coleoptile is, then, no more closely related to the scutellum than is that of the leaf above it. These facts would seem to indicate that the coleoptile bears the same relationship to the axis as the later leaves, except for the smaller number of coleoptile traces.

The internode between the coleoptile and the next foliage leaf (fig. 67) differs from the higher internodes of the axis (fig. 68) in about the same respects as the corresponding structures in maize and oats. Its bundles are not so sharply defined as are those of the higher internodes, and some transition between the exarch and the endarch xylem arrangement may be observed. In general, it more closely resembles the higher internodes than does that short interval in the axis between the level of divergence of the coleoptile and that of the scutellum. In contrast to the conditions in maize and oats, this internode is the principal elongating region of the seedling axis.

COLEOPTILE.—The origin of the vascular system of the coleoptile of wheat has been described. In transverse section its anatomy is so similar to that of the coleoptile of oats that no further description is necessary (figs. 54, 55). In contrast to the coleoptiles of maize and oats, however, that of wheat is not elevated (or only slightly so, as already pointed out) by a growth of the elongating axis, but instead, itself sheathes the elongating axis (figs. 35, 50). The coleoptile has been observed to reach a length of more than 4 inches. Its elongation depends on the depth of planting. PERCIVAL (17) reports the presence of from two to as many as six vascular bundles in the coleoptile of *Triticum dicoccum*. These bundles give the coleoptile distinct leaf-like characteristics, although it possesses no distinguishable midrib or blade.

LATER FOLIAGE LEAVES.—Structurally the leaf of wheat (fig. 56) is so similar to that of oats that no further description is necessary.

Discussion

That the scutellum alone is the cotyledon of the Gramineae is highly probable in the light of this investigation. This opinion is based on evidence afforded by its relation to the embryonic axis, its vascular system, and its abundant parenchyma. Physiologically it

functions as a storage and absorptive organ. The scutellum will hereafter be considered the cotyledon. The so-called "ventral scale" of the cotyledon, when present, may be interpreted as the ligule of the cotyledon, as pointed out by STRASBURGER (**24**).

The principal opposing view, held by GOEBEL, SARGANT and ARBER, and others, is that the scutellum together with the coleoptile constitutes the cotyledon, the coleoptile representing the cotyledonary sheath. Such an interpretation is based partly on the consideration that the vascular system of the scutellum and that of the coleoptile are intimately related and are distinct from the vascular system of the parts of the plant above them. As GOEBEL (**11**) states: "the bundles of the coleoptile may be considered as branches of that which enters the scutellum." Or, according to SARGANT and ARBER (**18**): "the scutellum trace ceases to exist at the first node, but each of its two halves maintains its identity within one of the two coleoptile traces." It has been shown in the present paper that such is not the case.

In the seedling of *Avena*, for example, the scutellum bundle extends into the axis and continues upward in the cortex to the level of divergence of the coleoptile. At this level it forms part of the stele and turns downward within it. At this same level, two branches diverge from it which extend first laterally and then upward in the axis (figs. 37, 40). As they extend upward each branch in turn diverges, and the outer divergences, one on either side, are coleoptile traces (fig. 40). Some of the traces of the leaves distal to the coleoptile are derived from the diverged scutellum bundle in the same manner as are those of the coleoptile. In addition, the midrib of the leaf above the coleoptile originates in part from the scutellum bundle. In the seedling of *Zea mays* the coleoptile traces are also divergences from bundles that continue upward in the axis (fig. 39). The origin of the bundles from which the coleoptile traces diverge is too variable and too difficult to follow for any exact statement, although it seems certain that at least part of their vascular tissue can be traced to the scutellum. The situation in *Triticum* (fig. 41) is similar to that in *Avena*, although the interval in the axis between the scutellum and the coleoptile is usually not elongated. From this evidence it can be seen that the coleoptile traces bear no separate

and distinct vascular relationship to the scutellum. The scutellar bundle diverges, at least in part, into traces of two or more leaves distal to it. This evidence favors the interpretation that the coleoptile bears a similar vascular relationship to the axis that any of the early leaves above the cotyledon (scutellum) bear to it.

If the coleoptile were interpreted as being the cotyledonary sheath and the equivalent of a ligule or a pair of fused stipules, as has been held by WORSDELL and others, it would not be expected that the cotyledon would be borne at one level on the axis and its ligule or fused stipules at another, as occurs in *Avena* and *Zea*. The coleoptile should appear in the same relation that a ligule or a pair of stipules would bear to a foliage leaf, and be borne at the same node. As for WORSDELL'S argument that the coleoptile is a ligule formed by the union of two stipules (based on the presence of the two vascular bundles which are commonly present in the coleoptile of *Zea mays*), I have previously shown that the coleoptile may possess from two to several bundles, thus indicating that it is not a double structure. There is no anatomical evidence that the coleoptile is a cotyledonary sheath, of either single or double nature.

This evidence, based particularly on the origin of the vascular supply and the anatomy of the coleoptile, would indicate that the coleoptile is the first independent leaf above the cotyledon, or the second leaf of the plant, and in consequence, not a cotyledonary sheath. Further support of this conclusion is given by the fact that buds are present in the axils of the coleoptiles of maize, oats, and wheat, which leaves little doubt as to the leaflike nature of the coleoptile.

It has been held that on the basis of origin the coleoptile must be part of the cotyledon. Such a conclusion might be based upon observations on the development of the embryo, because the coleoptile does arise from the lateral face of the embryonic tissue which later differentiates into the cotyledon.

This leads to the question of whether the cotyledon itself is a lateral or terminal structure. It is "terminal" if conclusions are drawn only from early embryogeny. Observations on the mature embryo, however, show the remains of the suspensor attached to the base of the coleorhiza (WEATHERWAX **28**). This appears to be definite evidence

that the embryonic stem tip is terminal, and the cotyledon a lateral structure. The fact that the embryonic stem tip appears to arise from the lateral face of the immature cotyledon need not be construed as meaning that the cotyledon is terminal. A more logical explanation, in view of the position of the suspensor in the mature embryo, would be to consider the origin of the growing point of the stem as having been delayed in development until after the cotyledon had attained considerable size. In fact, in many dicotyledonous plants the cotyledons are often nearly mature before there is an indication of the presence of a growing point for the epicotyl, as for example, in *Capsella*. For these reasons, the cotyledon in grasses is interpreted as a lateral structure, and the growing point of the stem as a terminal one. The origin of the coleoptile is associated with the origin of the stem tip, and the coleoptile is, therefore, no more a part of the cotyledon than is the growing point of the stem itself. The coleoptile must, then, be interpreted in the light of its later development. The fact that the coleoptile is borne on the axis with a vascular relation to this axis similar to that of the later leaves makes it unlikely that it is part of the cotyledon.

The usual objections to interpreting the coleoptile as the second leaf of the plant are, first, that it is not alternate distichous with the cotyledon; and second, that it is not differentiated into sheath and blade as are the later leaves. These points do not seem to offer serious difficulties when one considers the number of cases in which the early leaves of a plant are entirely different from its later leaves, both in form and arrangement.

The epiblast is present in oats and wheat, but does not occur in maize. It has been interpreted by various investigators as a rudimentary second cotyledon. Such an interpretation is untenable because no vascular connection with the stele has ever been observed. The fact that it is not universally present in grasses, together with the fact that it has no vascular system, and arises from the coleorhiza (as pointed out by CANNON and SOUÈGES), makes the epiblast appear to be of no more morphological significance than such a structure as the peg of the Cucurbitaceae. The conclusion of ARBER (1) and others that the epiblast bears essentially the same relation to the cotyledon that the auricles bear to the blade of the foliage leaf was

retracted (**2**) when she reiterated her earlier opinion (**18**) that it is of little significance.

The interval of the axis between the scutellum and the coleoptile, when obvious, has been variously interpreted: (1) as an elongated node between parts of a cotyledon (first named "mesocotyl" by CELAKOVSKÝ); (2) as a unique structure that has resulted from a fusion of the cotyledonary stalk with the hypocotyl; and (3) merely as an internode.

The first interpretation can be dismissed because it is unlikely that the structure in question could be an elongated node in light of the present evidence which substantiates the view that the coleoptile is an independent leaf, the second of the plant.

The second view, held by SARGANT and ARBER (**18**), that the "mesocotyl" is neither a node nor an internode, but a unique structure such as described, may be of value in attempts to establish phylogenetic relationships between the grasses and some other monocotyledons, but other interpretations of it are equally plausible.

From the evidence in hand, there are two principal objections to the theory. First, if this interpretation be true, elongation of the embryonic axis would start in the same relative position in all types, regardless of the degree of fusion. As pointed out, however, this is not the case. The presence of the cortical bundle in the elongating axis of *Avena*, and its absence in *Zea*, result entirely from the specific location of the first meristematic region (figs. 18, 19, 25, 27). Second, such a fusion would not account for the vascular plate occurring at the level of divergence of the cotyledon in maize, oats, and wheat. This zone marks the cotyledonary plate anatomically, and it would be the first node of the plant as long as it exists, regardless of peculiar subsequent developments and of elongation in unusual places.

SARGANT and ARBER have further attempted to support their fusion theory by pointing out that the coleoptile bundles appear double and apparently have a double origin, that is, that one half of each arises from the cotyledonary bundle, continues upward in the coleoptile, then turns back on itself and continues downward in the stele. They illustrate the possible origin of this condition by diagramming an "imaginary type." There is no anatomical evidence that each coleoptile bundle is composed of two laterally fused bun-

dles; instead, the part apparently derived from the stele is of the same origin as that from the cotyledonary bundle, because the cotyledonary bundle simply turns downward within the stele at the level of divergence of the coleoptile. Each coleoptile bundle is therefore of single origin, and is not composed of two laterally fused bundles. This evidence confirms the statement of BUGNON (5):

> Neither the direct observations of certain facts which would conform to the hypothesis (curve at the top of the coleoptilar bundles) nor the comparative anatomical study of lateral fusions of fibrovascular bundles, nor the ontogenetic study of cotyledonary development can permit us to accept the theory of the two English authors.

If the scutellum is the cotyledon and the coleoptile the second leaf of the plant, as the evidence in this study would indicate, then the third view, that the structure in question is an internode, is the correct one. According to this interpretation, the level of divergence of the cotyledon from the axis is the first node; that part of the axis immediately above the first node must of necessity be the first internode; the point of divergence of the coleoptile marks the second node; and the portion of the axis above this is the second internode.

Upon germination of the embryos of maize and oats, elongation of the axis begins in the first internode (figs. 19, 27). The second and subsequent internodes, sheathed by the coleoptile, are elevated to or near the surface of the soil. In the case of wheat, however, elongation is slight. The second node remains underground. The greatest elongation takes place in the second internode, elevating the third and subsequent nodes to the surface of the soil (fig. 35). The fact that the first internode in wheat often fails to elongate at all, in no way implies that it is not an internode.

The presence of the cortical bundle in the first internode of oats, and the fact that it first becomes part of the stele at the level of the coleoptilar node, have been construed as meaning that the vascular plate of the cotyledon is at the level of divergence of the coleoptile. As previously pointed out, there is a very definite vascular plate at the level of divergence of the cotyledon. The level of this cotyledonary plate remains the same regardless of later developments. The presence of the cortical bundle may be attributed entirely to the location of the first meristematic region of the axis (figs. 25–27).

The type of development of the xylem in the first and second internodes of maize, oats, and wheat has been pointed out. Whether or not any marked elongation of the first internode has taken place, both exarch and endarch xylem groups are present in it. The structure bears resemblance both to what is often referred to as typical root structure and stem structure. The second internode is more nearly typically stemlike, but still shows considerable transition in arrangement. This transition may continue even in the third and to some extent in the fourth internode. This condition of gradual transition through the lower internodes may be found in dicotyledonous plants, such as *Pisum* and others with hypogeal cotyledons. It may be regarded as supporting evidence in the interpretation that the interval in the axis between the level of divergence of the cotyledon and that of the coleoptile is the first internode, which displays a very markedly intermediate condition between root and stem structure.

Summary

1. The scutellum in maize, oats, and wheat is the cotyledon. The "ventral scale" of the cotyledon, when present, may be interpreted as its ligule. The epiblast, when present, cannot be considered a rudimentary cotyledon; it probably has little morphological significance. These interpretations hold also for other grasses examined, including barley, rye, and rice.

2. The coleoptile is homologous with a foliage leaf, and is the second leaf of the plant, the leaf distal to it being the third leaf of the plant.

3. In maize and oat seedlings, the elongated structure between the cotyledon and the coleoptile is the first internode of the axis. The term "mesocotyl" as applied to this structure is meaningless. In wheat, the corresponding structure, although not elongated, is likewise the first internode of the axis. This holds true for other Gramineae that develop similarly to maize, such as oats and rice, and those that develop like wheat, such as barley and rye. The principal elongating structure in the young wheat axis is the second internode. It is always sheathed by the coleoptile.

4. There is no hypocotyledonary region of "transition" in the grasses. The transition is confined to stem structure. It starts and

takes place largely in the vascular plate at the first node, and in the first internode. The transition continues in the second internode, and to some degree even in the third and fourth internodes.

5. The three morphological types distinguished by VAN TIEGHEM are fundamentally one type, appearing differently upon development because of the difference in location of the meristematic region in the first internode. These differences often show in the mature embryo, before germination begins. The similarity of the vascular skeletons has been pointed out.

The writer expresses great gratitude to Professor E. J. KRAUS, who suggested the problem and has made many constructive criticisms during the progress of the investigation. Professor C. E. ALLEN has also been very helpful.

DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

[*Accepted for publication March 9, 1929*]

LITERATURE CITED

1. ARBER, A., Leaves of Gramineae. BOT. GAZ. **76**:374–388. 1923.
2. ———, Studies in the Gramineae. III. Outgrowths of the reproductive shoot, and their bearing on the significance of lodicule and epiblast. Ann. Botany **41**:473–488. 1927.
3. AVERY, G. S. JR., Coleoptile of *Zea mays* and other grasses. BOT. GAZ. **86**: 107–110. 1928.
4. BRUNS, E., Der Grasembryo. Flora **76**:1–33. 1892.
5. BUGNON, P., La feuille chez les Graminées. Mém. Soc. Linnéenne de Normandie. **21**:108. 1921.
6. CANNON, W. A., A morphological study of the flower and embryo of the wild oat, *Avena fatua* L. Proc. Calif. Acad. Sci. ser. III Bot. **1**:329–362. 1900.
7. CELAKOVSKÝ, L. J., Über die Homologien des Grasembryos. Bot. Zeit. **55**: 141–174. 1897.
8. CLOS, D., Du collet dans les plantes, et de la nature de quelques tubercles. Ann. Sci. Nat. Bot. III. **13**:5–20. 1849.
9. COULTER, J. M., The origin of monocotyledony. II. Monocotyledony in grasses. Ann. Mo. Bot. Gard. **2**:175–183. 1915.
10. DEBARY, A., Comparative anatomy of the phanerogams and ferns (Eng. Transl. by F. O. BOWER and D. H. SCOTT). Oxford. 1884.
11. GOEBEL, K., Organography of plants. Part II (Eng. Transl. by I. B. BALFOUR). Oxford. 1905.

12. GRIS, A., Recherches anatomiques et physiologiques sur la germination. Ann. Sci. Nat. Bot. V. **15**:70–83. 1864.
13. HEGELMAIER, F., Zur Entwicklungsgeschichte monokotyledoner Keime, nebst Bemerkungen über die Bildung der Samendeckel III. *Triticum vulgare* L. Bot. Zeit. **32**:657–671. 1874.
14. DE JUSSIEU, ADRIEN, Sur les embryons monocotylédonés. Ann. Sci. Nat. Bot. II. **11**:341–361. 1839.
15. LESTIBOUDOIS, T., Phyllotaxie anatomique, ou recherches sur les causes organiques des diverses distributions des feuilles. Ann. Sci. Nat. Bot. III. **10**:136–189. 1848.
16. PERCIVAL, J., The wheat plant. New York. 1922.
17. ———, The coleoptile bundles of Indo-Abyssinian emmer wheat (*Triticum dicoccum* Schübl.). Ann. Botany **41**:101–105. 1927.
18. SARGANT, E., and ARBER, A., The comparative morphology of the embryo and seedling in the Gramineae. Ann. Botany **29**:161–222. 1915.
19. SARGANT, E., and ROBERTSON, A., The anatomy of the scutellum in *Zea mays*. Ann. Botany **19**:115–124. 1905.
20. SCHACHT, H., Lehrbuch der Anatomie und Physiologie der Gewächse. **1**:323–334. 1859.
21. SCHLEIDEN, M. J., Sur la formation de l'ovule et l'origine de l'embryon dans les Phanérogames. Ann. Sci. Nat. Bot. II. **11**:129–141. 1839.
22. SCHLICKUM, A., Morphologischer und anatomischer Vergleich der Kotyledonen und ersten Laubblätter der Keimpflanzen der Monokotylen. Bibliotheca Botanica Bd. 6. 1896.
23. SOUÈGES, R., Embryogénie des Graminées. Développement de l'embryon chez le *Poa annua* L. Compt. Rend. Acad. Sci. Paris **178**:860–862. 1924.
24. STRASBURGER, E., Textbook of botany (Eng. Transl. by W. H. LANG). 5th Eng. ed. London. 1921.
25. TOOLE, E. H., The transformations and course of development of germinating maize. Amer. Jour. Bot. **11**:325–350. 1924.
26. VAN TIEGHEM, P., Observations anatomiques sur le cotylédon des Graminées. Ann. Sci. Nat. Bot. V. **15**:236–276. 1872.
27. ———, Morphologie de l'embryon et de la plantule chez les Graminées et les Cypéracées. Ann. Sci. Nat. Bot. VIII. **3**:259–309. 1897.
28. WEATHERWAX, P., Position of scutellum and homology of coleoptile in maize. BOT. GAZ. **69**:179–182. 1920.
29. ———, The story of the maize plant. Chicago. 1923.
30. WORSDELL, W. C., The morphology of the monocotyledonous embryo and of that of the grass in particular. Ann. Botany **30**:509–524. 1916.

RESPIRATION IN STRAWBERRY FRUITS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 399

ARTHUR R. GERHART

(WITH FOUR FIGURES)

Introduction

Strawberry culture in the United States has become an important industry. In commercial value it has even taken rank with the white potato, as indicated in the United States Department of Agriculture Year Books. While Tennessee retains the leading position in strawberry culture, it is now followed closely by Louisiana. In Chicago the first shipments received come from Florida in December. By February the source of supply has shifted to Louisiana. From that time on until August there follow in rapid succession the producing areas of Mississippi, Arkansas, Tennessee, southern and northern Illinois, Wisconsin, Michigan, and even Montana as points of origin. Other consuming centers are of course supplied by a commerce equally complex.

Because the market and the point of production are often separated by great distances, and the fruits are highly perishable, the obstacles to commercial horticulture and market gardening (which are the causes of a really stupendous annual financial loss) are obviously of special severity in the strawberry industry. The present investigation was undertaken with the thought that it might help to throw light on the problems of transportation and storage, and at the same time have considerable purely scientific interest.

Botanical literature is replete with studies of the respiration of plant tissues. Most of these, however, deal with the seeds or with the vegetative parts, such as shoots and leaves of plants. Study of fruit respiration has been by comparison a neglected field. Also the usual procedure has been to measure the CO_2 output rather than the O_2 consumption, and to employ it as the sole index of respiration. Really, both CO_2 and O_2 have to be measured in order to arrive at

anything more than an approximate knowledge of the respiration process, as has been pointed out by HARRINGTON (**16**).

Source of material

The strawberries used were purchased in the Chicago market during the months from December, 1927, to July, 1928, and had been refrigerated to Chicago. The variety chiefly used was the Missionary, grown in both Florida and Louisiana. Some work was also done with the Gibson and the Brandywine. With regard to the effect of refrigeration on the respiratory rate, it may be stated at once that the present study is one concerned with strawberries that have undergone rapid transit and brief storage at temperatures of from 10° to 16° C.

Although fresh material was invariably secured from day to day, it was not noticed that a preservation of from two to three days in the ice-box had any noticeable effect upon the respiratory rate of a sound and uninjured berry. What the character of respiration of the freshly picked fruit is, this paper cannot attempt certainly to answer; although, from the results of some preliminary work with freshly collected berries in the autumn of 1927, it is suspected that it is not markedly different from the data here given. Nor are we dealing here with "normal" respiration which could only be determined by experimenting with undisturbed ripe fruits still attached to the plants.

Fresh-looking uninjured fruits were always selected from the boxes for use. The green calyx was not removed from the berry.

Apparatus

Since the KRAJNIK (**19**) respirometer has not previously been used in this country in work with plants, and only once, to the writer's knowledge, in experimental work of any kind (ALLEE **1**, in his investigations upon isopods), a description of it (fig. 1) and its operation may be given here.

The apparatus consists essentially of a closed system of capillary tubing terminated at either end by a glass bottle. The bottle at the left contains the specimen being studied, while that at the right is empty and serves as a control. The advantage of a closed system, of course, is that changes of barometric pressure are obviated.

The middle of the tube is curved to form a loop and is set against a glass mirror bearing a millimeter scale. Into this part of the apparatus is introduced a column of kerosene colored with Sudan III.

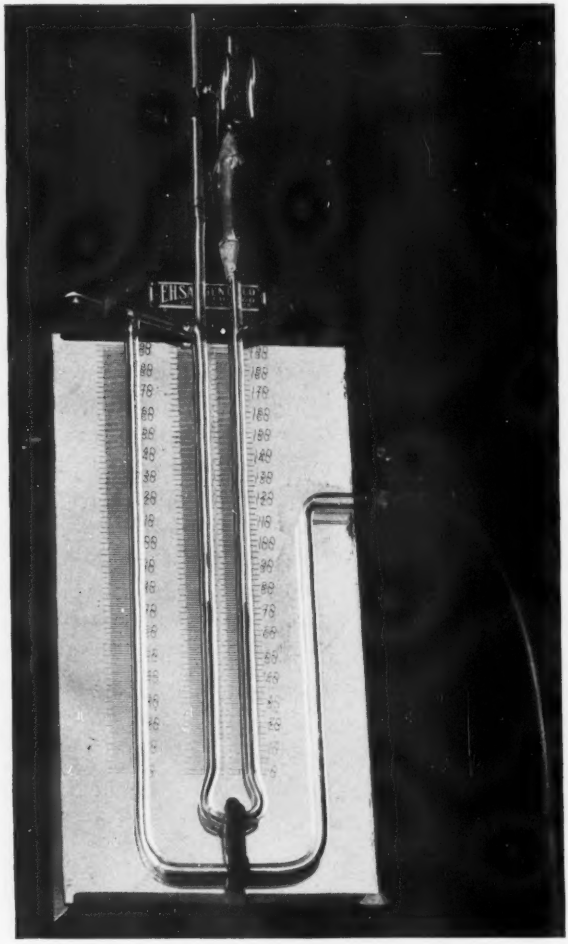


FIG. 1.—Krajnik respirometer: tube containing kerosene in center; outer tube contains mercury.

This liquid moves up or down with reference to the scale in response to changes of gaseous pressure in the bulbs at the ends of the tube. In the apparatus as originally used by KRAJNIK a single drop of liquid was employed instead of a column, as used in the present study. Connecting with the tube containing the colored kerosene on the left side of the system is a second capillary tube, which curves around below, forming a U, and which is made to contain mercury. The bottles are round-bottomed with a capacity of 75–80 cc. They are fitted with hollow ground glass stoppers and sealed with mercury (fig. 2). At a point on the inside surface of the glass stopper a small glass rod is attached which extends down into the bottle when the stopper is in place. At the lower end of this glass rod is a cup with a capacity of about 1 cc. to hold the carbon dioxide absorbent.

FIG. 2.—Container for respiring fruits

The entire apparatus is mounted on a wooden frame so that it may be placed in position over a constant temperature tank with the bottles completely submerged in the water. Two rubber connections and a single piece of glass tubing are necessary to connect each bottle to its end of the respirometer. These rubber connections

need not constitute more than a fraction of an inch in the length of the entire system, however, if the glass capillary tubes are just long enough to permit the proper submergence of the bottles. Moreover, as the CO_2 was absorbed in the experiment, and it is not known that oxygen effects any very rapid union at low temperature with rubber, the use of thick-walled rubber tubing cannot be objected to.

The temperature of the water bath may be lowered by the use of ice, or raised by the heat from two large electric bulbs on a direct current. It may be maintained at any desired point by the use of a third bulb in a "make and break" circuit, with a well of mercury with which a platinum pin is adjusted to make contact at the desired temperature. The constant temperature tank used has inside dimensions of $28'' \times 28'' \times 13''$, and consequently holds a volume of water whose temperature is not readily changed by extraneous causes. Actually, modification of the temperature rarely amounted to as much as 0.5° C. during the course of any series of determinations, and was practically nil at 25° or above. In order to equalize the temperature of the water, it was kept constantly in motion by a stirring rod run by an electric motor.

Operation

When, as a result of changes of pressure in one of the bottles, the kerosene moves either up or down with reference to the scale, it may be brought back to its original position by forcing the mercury farther up the vertical tube, or withdrawing it as the case may be, until the levels of the two columns of kerosene are back in their first positions. But this can only have been accomplished through a restoration of the original pressures. Since the volume of gas, and its temperature as well, in the right half of the system have not changed, the change in volume and hence of pressure must have been a result of the activities of the plant tissues confined in the bottle to the left. The amount of this volume change is then directly indicated from the scale set behind the mercury tube. By thus raising or lowering the mercury column, a manipulation readily accomplished by pressure of the fingers on the rubber tube attached to the free end of the mercury tube and which thus acts as a sort of bulb, readings may be successively made at any moment or interval

of time desired; or, at the close of each period of observation the stopcocks above (which admit air from the outside directly into the system) may be opened, thus restoring the pressure in the bulbs to that of the atmosphere, and closed again at the beginning of the next observation.

The advantage of this apparatus over all others known to the writer is its extreme sensitivity. It is possible with it to measure directly the respiration during a minute or even less time of one single strawberry.

For the purpose of greater clarity a single computation may be presented here. The diameter of the bore of the tubing is determined by careful measurement with a micrometer. From this the cross-section area is readily calculated and is found for the instrument used to be 0.02175 sq. cm. This value is used in all the computations, and is multiplied by the value in linear centimeters obtained from reading the instrument in order to calculate the volume change in cubic centimeters.

In one instance a strawberry weighing 4.2 gm. was used, the temperature of the water bath being 25° C. At 10:40:30″ A.M., the level of the mercury being at 3 mm., the stopcocks above were closed simultaneously. The kerosene begins at once to rise in the left side of the instrument. Now the mercury is raised to counterbalance this change, and when the tops of the kerosene columns are again exactly level, the height of the mercury column is read and the exact time noted. The mercury is at the 42 mm. point and the time is 10:45. Then 4.2 cm. -0.3, or 3.9 cm. $\times 0.02175 = 0.084825$ cc., the total volume of gas used. But the experiment has extended through four and one-half minutes; therefore $0.084825 \div 4.5 = 0.019072$ cc. O_2 was consumed in one minute. But the strawberry weighs 4.2 gm.; therefore $0.019072 \div 4.2$ or 0.00454 cc. is the amount of oxygen consumed per gram of strawberry per minute of time, or 272.4 cc. per kilogram hour.

It was found by inserting a thermometer into a strawberry taken from the refrigerator, that several hours were required for it to reach room temperature; consequently berries intended for experiment were placed in an extra bottle and kept in the water bath for some time prior to their being placed in the respirometer for observa-

tion. It was discovered from blank trials that it was necessary to put the bottles in place and set the stirring rod in motion for about half an hour before observations were started, so that the temperature of the parts of the apparatus might be equalized. In order to facilitate this, the small cups after being filled with the 40 per cent NaOH solution (which was used to absorb the CO_2 produced by the strawberries) were dipped for a moment into the water in the tank before being placed in the bottles. It was found of great importance to remove droplets of moisture which from any cause formed in the capillary tubing. It was likewise found that air currents set up in the room as the result of open doors and windows were to be avoided.

It may be well to point out that changes of volume of the gas in the bottle containing the berry cannot be determined practically by a direct reading of the change in the levels of the columns of kerosene. Were this attempted, a number of corrections to the values as obtained would be necessary. The first of these would be for the negative pressure in the gas in the control bottle. For as the kerosene moves from right to left a partial vacuum is set up in the control bottle. This would result in a reduced pressure, allowance for which would have to be made. Also, as the kerosene rises in one arm (the left) of the tube containing it, and falls in the other (the right), the weight of a column of kerosene whose height is the difference between the two levels acts as a negative pressure on the gas in the bottle containing the berry. Finally, it will be apparent that the loop of the capillary tube offers a considerable amount of friction to the passage of a tenuous column of liquid around it, and that once the loop is passed an accelerated movement will result, so that the rate of movement of the kerosene will be much faster during the latter part of a period than in the first part. By computation, it was found that the observed movement of kerosene corresponded to only one-tenth of the calculated change in volume when corrections were applied for the first two of these factors; and it would be difficult to calculate quantitatively the effect of the last one. Subsequent confirmation of the accuracy of the magnitude of this correction was afforded by the use of the proper method, and all of the difficulties and some others which need not be mentioned were avoided by its adoption.

Results

Oxygen consumed in respiration at different temperatures

The first object was to measure initial rates of respiration of the strawberry at different temperatures. As it would not be feasible to include here ali the data obtained, some series are presented as samples in table I. The table shows consumption of O_2, the CO_2 being absorbed by NaOH solution, as previously mentioned.

TABLE I

Amounts of oxygen consumed in respiration at different temperatures (Missionary variety)

Temperature 5° C., weight of berry 7.58 gm.		Temperature 20° C., weight of berry 6.60 gm.		Temperature 25° C., weight of berry 5.856 gm.		Temperature 36.5° C., weight of berry 5.551 gm.	
Duration of experiment (minutes)	O_2 consumed per gm. per minute (cmm.)	Duration of experiment (minutes)	O_2 consumed per gm. per minute (cmm.)	Duration of experiment (minutes and seconds)	O_2 consumed per gm. per minute (cmm.)	Duration of experiment (minutes)	O_2 consumed per gm. per minute (cmm.)
38.....	0.7250	16.....	2.840	2:30...	4.434	6.....	8.290
24.....	0.7650	10.....	3.130	2:30...	4.434	8.....	9.207
18.....	0.6700	6.....	3.185	2:16...	4.508	6.....	8.881
51.....	0.7257	11.....	3.325	1:50...	4.238	7.....	8.956
15.....	0.6886	12.....	3.050	4: 0...	4.671	7.....	8.956
14.....	0.7173	18.....	2.966	10:10...	4.422	6.....	9.080
18.....	0.7010	18.....	2.929	15:10...	4.032	5.....	8.776
		15.....	2.966	15:15...	4.344	7.....	9.120
		20.....	3.000	15:10...	4.320		
		16.....	2.700	14:55...	4.380		
		10.....	2.768	9: 0...	4.166		
		14.....	2.871	11: 0...	4.220		
				12: 0...	4.240		
				12:45...	4.312		
Average O_2 consumed per gm. per min.	0.7132	.....	2.977	.....	4.337	.....	8.908

The results obtained from Missionary berries are summarized in table II. All of these data are from observations made upon the berries at once after leaving them in the apparatus only long enough to attain the proper temperature ($\frac{1}{2}$–$2\frac{1}{2}$ hours). The figures therefore represent the initial respiration of berries at the temperatures indicated. The 191 trials made have an average length of 10–15 minutes.

Fig. 3 shows a respiratory curve constructed from these data. The significance of the figures when converted into market units may be seen readily. A box of strawberries of this variety as sold at the grocery weighs 661 gm. or 1.46 pounds, without the box. Eighty of these "quarts" are packed into a freezer or small refrigerator, of which about forty are packed into a car. At 20° C. (70° F.) these berries would consume 13.35 cubic feet of oxygen per hour; at 10° C. (52° F.) they would consume 5.34 cubic feet. If this oxygen were used in the oxidation of glucose, sufficient heat would be produced

TABLE II

RATES OF RESPIRATION OF MISSIONARY STRAWBERRY

TEMPERATURE OF TEST (° C.)	CUBIC MILLIMETERS OF O_2 CONSUMED PER GM. OF BERRY PER MINUTE	NUMBER OF TRIALS
5	0.7132	7
10	1.1936	12
15	1.8000	12
20	2.9775	12
25	4.4920	74
30	6.3020	19
35	7.6796	18
36.5	8.6390	15
37.5	8.0430	8
40	4.7108	13

to melt 318.7 pounds of ice in the first instance, and 127.5 pounds in the second.

In Louisiana, the berries are packed in pint boxes, weighing about 500 gm. or 1.1 pounds exclusive of the box. Twenty-four of these are put into a crate, of which about 800 are packed into a car. At 20° C. these berries would consume 64.87 cubic feet of oxygen per hour; at 10° C., 26 cubic feet per hour. The heat produced in these oxidations would melt 1548.5 pounds of ice in the first instance and 620 in the second.

From Tennessee come carloads of 420 cases of 24 quarts each. These also weigh about 500 gm. or 1.1 pounds. At 20° C., 31.78 cubic feet of oxygen would be required by them per hour; at 10° C., 12.7 cubic feet. The heat produced if these amounts of oxygen were used to oxidize glucose completely would melt 758 and 303 pounds of ice respectively.

Temperature coefficient of respiration

It may be seen from table II that the relations between the values for the respiration at 5° and 15°, that between those for 10° and 20°,

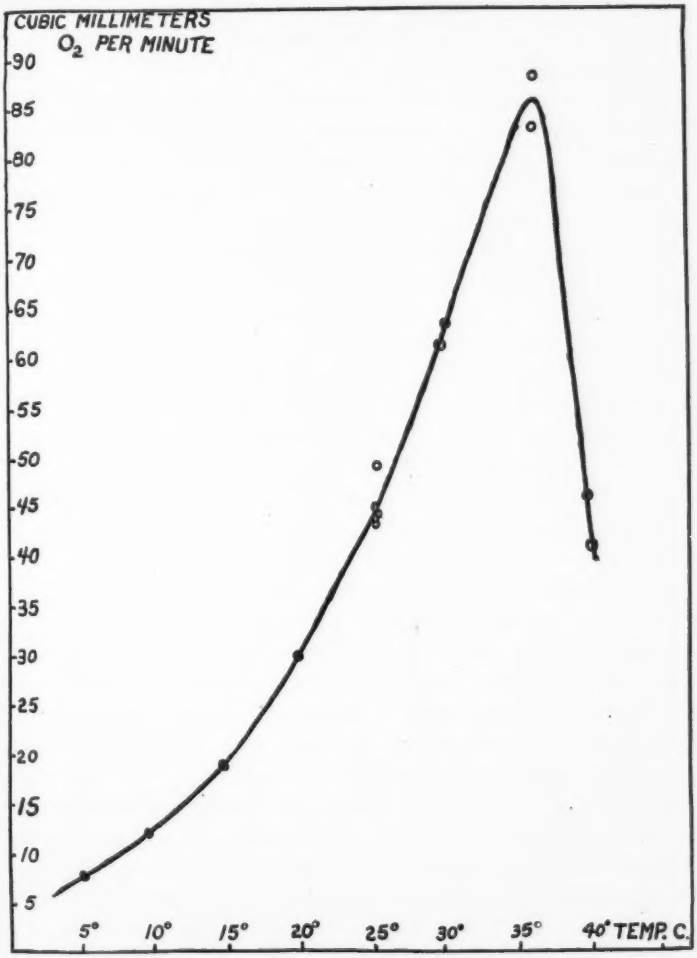


Fig. 3.—Graph showing respiration of Missionary strawberry

and also the relation between those for 15° and 25° are almost exactly (and within a very small error) what they would be assum-

ing a value of 2.5 for the Q_{10} of strawberry respiration. This result is quite in accord with the work of other students of respiration. CLAUSEN (9) found a Q_{10} value of 2.5 for wheat, lupine seedlings, and syringa flowers between 0° and 20°. GORE (15) found an average coefficient for a rise in temperature of 2.377 ± 0.024 in his studies upon the respiration of fruits.

Above the 25° temperature, however, it is impossible to discover any consistent ratio in the data given (table II) for the strawberry. The reason for this is doubtless to be found in the fact that the cell membranes cannot be penetrated quickly enough by oxygen to prevent the formation of a deficit of this substance. This in itself would introduce a serious limiting factor to a continued geometrical rate of increase in the rate of a chemical reaction depending upon these materials. Further, when aerobic respiration becomes impossible, anaerobic respiration is initiated, resulting in the production of alcohol and various organic acids. These in turn, through their effect upon the protoplasm or their inhibiting effect upon the enzymes concerned, would introduce a further complication, so that is is impossible to discover any consistency with the Van't Hoff rule at the higher temperatures. It is not likely that the amounts of plastic materials available for oxidation would play the part of limiting factors in the case of respiration of fruits, as it might be expected to do, for example, with that of leaves.

CROZIER (10), in his efforts to identify the chemical processes in living matter, proposes to do so through the discovery of characteristic "critical temperature increments" which are identical in many different biological processes. The values which he finds (11500, 16100, and 16700 calories) have much the same significance as respiratory coefficients, in that they are expressions of regularity in the increase of the velocity of vital processes. He is compelled to assume a similarity between biological processes and a monomolecular reaction, which involves precisely the same obstacle to the successful application of the system of respiratory coefficients to rates of acceleration of biological respirations at higher temperatures.

MAXIMUM RESPIRATORY TEMPERATURE

It is common knowledge that plant activities are at their maximum rate at relatively high temperature. It is also known that from

the peak of activity maintained over a short interval of time the rate declines, although the temperature remains constant. Thus LEITCH (20), in studies upon the growth of *Pisum sativum*, recognized in addition to the customary absolute minimum, optimum, and maximum points, an additional fourth one, the maximum rate at a temperature point in which the time factor is likewise given consideration. BLACKMAN'S (6) paper is also insistent on this point. Respiratory activity in the strawberry follows this rule. It will be seen from fig. 2 that the maximum rate of respiratory activity is in

TABLE III

CUBIC MILLIMETERS O_2 CONSUMED PER MINUTE PER GM. OF STRAWBERRY

TEMPERATURE (° C.)		NUMBER OF TRIALS
30	Initial rate 6.302	19
	After 18 hours 5.304	4
	After 24 hours 3.51	6
35	Initial rate 7.6796	18
	After 18 hours 5.731	4
36.5	Initial rate 8.639	15
	After 2 hours 7.921	7
37.5	Initial rate 8.043	8
	After 2½ hours 7.688	9
	After 3 hours 7.2	8
	After 6 hours 6.8836	7
40	Initial rate 4.7108	13
	After 22 hours 3.25	12

the neighborhood of 35.5° C. But this rate is not maintained for very long, very quickly falling. Thus Missionary strawberries whose initial rate of respiration at 30° C., as already shown, is 6.302 cu. mm. per minute, becomes 5.305 in 18 hours' time and 3.51 at the end of 24 hours.

The rate established as the initial one at 35° falls more quickly in 18 hours from 7.679 to 5.731. While that discovered for the 36.5° (8.639) falls in two hours to 7.921. The rate for 37.5° (8.0) drops in 2.5 hours to 7.688, in 3 hours to 7.2, and in 6 hours it has become 6.8836. The rate at 40° (4.4119) likewise falls to 3.2519 in 22 hours. These results are summarized in table III, and expressed graphically in fig. 4.

It may be seen that the higher the initial rate the more rapidly it falls. The highest two rates (that is, those for 36.5° and 37.5°) at the expiration of three hours are less than the rate for 35°, and the rates for all the temperatures employed above 25° show a disposition to fall below the initial 25° rate, which was approximately 4.45 cu. mm. per minute (table I). Thus it appears that over a period of 6 or 8 hours the optimum rate would not be at 36.5° nor yet at 37.5°,

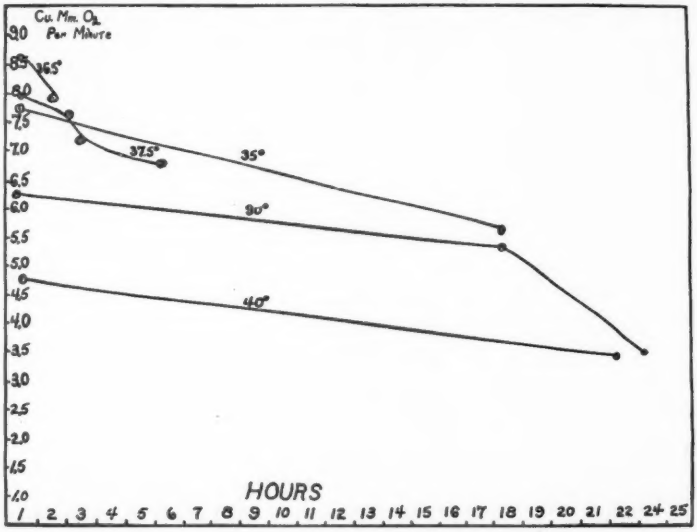


FIG. 4.—Curve of respiratory rate at temperatures indicated over periods of time (Missionary).

but rather at 35° and for longer periods it would be even lower. Thus accepting LEITCH'S definition of optimum as the temperature in which no time factor enters, it would probably be between 25° and 30° C.

The strawberry appears to suffer injury at higher temperatures. It is for this reason difficult to secure readings of the respiratory rate upon berries which appear at the close of the series of trials to be in normal condition.

Humidity factor in respiration

In the major part of the work here reported, no attention was paid to the factor of humidity as influencing respiration. What possible effect it might have was shown by the following experiment.

The work was done with the Gibson strawberry, grown in Wisconsin. Respiration rate was first determined for the berries as they were obtained on the market. The results of two series of determinations were as follows: first series of seven trials, aggregating 125 minutes, 2.505 cu. mm. of oxygen per minute; second series of six trials, aggregating 123 minutes, 2.508 cu. mm. of oxygen per minute. The readings were unusually consistent throughout.

Two lots of these berries were then placed in desiccators, the one containing $CaCl_2$, the other a beaker containing water. The desiccators were allowed to remain at the temperature of the laboratory for 24 hours. At the close of this period, a series of readings was made of the respiration of the berries in the $CaCl_2$ desiccator. In this experiment, as with the one later described, of the berries set in the $CaCl_2$ desiccator, $CaCl_2$ was also put in the bottle in which the berry respired while under observation. The results of eleven series of observations, extending over 184 minutes, gave an average value of 2.780 cu. mm. of O_2 consumed per minute per gram of berry.

At the expiration of 48 hours respiratory activity was again observed. The strawberries in the $CaCl_2$ desiccator, as the results of seven tests of 123 minutes in duration, were shown to be respiring at the rate of 1.885 cu. mm. of O_2 per minute per gm. of berry, while those from the humid chamber were using 1.992 cu. mm. These latter results were obtained as a result of five measurements of 70 minutes' duration. These results are presented in table IV.

It appears from the figures that a temporary increase in the rate of respiration occurred as a result of the stimulus of dry air, and that this later fell off to a point slightly below that of the berries held in moist air. This is in accord with the work of Luthra (22) upon Conference pears. The results seem to justify the assumption that ordinary fluctuations of atmospheric humidity can be disregarded to a considerable extent as a factor affecting rates of respiration in the strawberry.

In a further effort to substantiate this conclusion, a determina-

tion was made of the actual water content within the berry after an exposure to dry air. The first method was that of placing weighed quantities of berries in the desiccator with $CaCl_2$, and contrasting their loss of weight with that of berries held in a humid chamber. Berries used were carefully selected for soundness, with no abrasions of the epidermis. Table V gives the results of these tests.

TABLE IV

EFFECT OF HUMIDITY UPON RESPIRATORY RATE OF GIBSON STRAWBERRY (ALL MEASUREMENTS AT 20° C.)

CONDITIONS	RESPIRATORY RATE EXPRESSED IN CMM. OF O_2 CONSUMED PER GM. OF BERRY PER MIN.	NUMBER OF TRIALS	TIME IN MINUTES
Newly purchased berries:			
First series	2.505	7	125
Second series	2.508	6	123
After 24 hours in desiccator containing $CaCl_2$	2.780	11	184
After 48 hours in desiccator containing $CaCl_2$	1.885	7	123
After 48 hours in moist chamber	1.992	5	70

TABLE V

LOSS OF WEIGHT OF MISSIONARY STRAWBERRIES IN DRY AND MOIST AIR

CONDITIONS	ORIGINAL WEIGHT OF BERRIES (GM.)	WEIGHT AFTER 17 HOURS (GM.)	AMOUNT OF LOSS IN WEIGHT (GM.)	PERCENTAGE LOSS IN WEIGHT	WEIGHT AFTER 50 HOURS (GM.)	TOTAL LOSS IN WEIGHT (GM.)	PERCENTAGE OF LOSS
Placed in desiccator containing $CaCl_2$	80.60	80.04	0.56	0.695	79.03	1.57	1.95
Placed in humid chamber	79.16	78.86	0.30	0.380	78.28	0.88	1.11
Difference	.....	.....	.....	0.315	.....	.....	0.84

Only those results obtained after the lapse of considerable time, for example, 17 hours, are regarded as especially significant, in as much as the slight difference between the weights at earlier periods might well be due to a loss of hygroscopic moisture from the surfaces of the berries. From these results, it appears that the epidermis of a

strawberry must be an exceedingly effective covering for the prevention of water loss. Therefore, insofar as dry air acting upon respiration of strawberries indirectly (by causing an undue water loss) is concerned, its effect is demonstrably small.

This decline in weight is not to be thought of as the rate at which boxes and crates of strawberries on the market lose weight. The decline is in reality considerably faster, owing to the presence generally of many diseased and injured fruits. This fact the following measurements will show:

FIRST MEASUREMENT

A "pint" box (box weighing 50 gm.) of Gibson strawberries was used just as received at grocery.

WEIGHT	GM.
Including box, Thursday, July 5, 5:00 P.M.	555.12
After storage in icebox, Friday, July 6, 3:20 P.M.	549.95
After storage in icebox, Saturday, July 7, 9:00 P.M.	534.00
After storage in icebox, Monday, July 9, 4:30 P.M.	505.00

Thus, in 4 days' time the strawberries had lost 10 per cent of their weight. At this time the berries were moldy and in bad shape. Upon picking them over, only 176 gm. of sound berries remained from the entire lot.

SECOND MEASUREMENT

A "quart" box of Florida-grown Missionary strawberries when received at the grocery (including the box in which they were packed, weighing 42 gm.) was used in these measurements, taken after storage at 10° C.:

WEIGHT	GM.
Thursday, March 15, 2:00 P.M.	703.0
Friday, March 16, 2:45 P.M.	681.0
Saturday, March 17, 2:00 P.M.	664.0
Monday, March 19, 11:00 A.M.	631.3
Tuesday, March 20, 2:00 P.M.	612.0
Wednesday, March 21, 1:00 P.M.	594.5

Again, in 4 days' time the berries had lost 10 per cent of their weight. At this time the moldy and decayed berries were removed, leaving but 214.3 gm. of sound ones.

A still further confirmation was sought by determining the percentage of moisture contained in strawberries as a result of exposure to desiccated air. The results are shown in table VI.

The data obtained in these tests may be compared with that of an analysis made upon berries as they arrived on the market. The duplicates showed 89.67 and 89.89 per cent of water loss, or an average of 89.78 per cent.

These results confirm the conclusion that water loss is very little greater in dry than in moist air, and that consequently the indirect effect upon respiration as a result of such water loss cannot be great. This conclusion is not vitiated by the results of KOLKWITZ (**29**),

TABLE VI

MOISTURE DETERMINATIONS WITH GIBSON STRAWBERRY AFTER RESPIRING IN MOIST AND DRY AIR

WEIGHT	FIRST SAMPLE (GM.)	SECOND SAMPLE (GM.)	AVERAGE (PER CENT)
After respiring 24 hours in desiccator	14.870	14.470	
After drying 12 hours in vacuum oven at 60° C.	1.608	1.5330	
Loss in weight as result of drying	13.262	12.9370	
Percentage loss	89.186	89.4050	89.2955
After respiring 24 hours in moist chamber	17.480	17.5000	
After drying 12 hours in vacuum oven at 60° C.	1.780	1.7312	
Loss in weight as result of drying	15.700	15.7688	
Percentage of loss	89.817	90.1670	89.9626
Difference in moisture content			0.6671

DUVEL (**12**), BAILEY and GURJAR (**4, 5**), and many others, who find that moisture accelerates respiration in seeds. In the respiration of seeds, moisture is doubtless a limiting factor.

Although over long periods of time considerable metabolic water is produced as a result of aerobic respiration, as BABCOCK (**3**) has shown, the amount of water contained in the plant tissues appears to be more or less carefully regulated. Thus MORSE (**25**), working with apples, finds a constant proportion of water and dry matter which existed over a length of time of several months, in spite of a continuous loss of water and decrease of weight. A dry weight determination made upon green strawberries showed percentages of moisture of 90.44 and 90.61 in the duplicates, the average being

90.525. This somewhat surprising result, showing a moisture content equal to that in the ripe berries, also the results upon ripe strawberries just given, together with the results of other analyses,[1] indicate not only that this condition of water equilibrium is maintained in the strawberry as in the apple, but also suggest that approximately the same amount of water exists in the fruit over the entire ripening period. In this connection, the findings of LOREE (21) are of interest. Experimenting with various fertilizer treatments of strawberries, he found that although the size of the berries, number of flower clusters, and nitrogen and sugar content are subject to variation, very little effect is noticeable on the moisture content of the fruit. Accordingly, save for a temporary effect of perfectly dry air in stimulating respiration, an influence which would not be likely to exist in practical marketing operations, the entire matter of water within and moisture without the berry ceases to be really significant in respiration. This fact, however, does not mean that humidity may not be an important factor in marketing losses from disease organisms or other types of physiological behavior.

RESPIRATION OF GIBSON STRAWBERRY

The only study upon the respiration of the strawberry which is available in the literature is that of GORE (15). His curve for the respiration of the Gandy variety is based upon three determinations. His results, translated into the units employed in this paper, are as follows:

2° C.	0.1283 cu. mm. CO_2 per gm. berry per minute
10.6° C.	0.405 " " " " " " " "
26.2° C.	1.405 " " " " " " " "

His respiration curve for Martin's New Queen's strawberry is determined by two measurements:

10.1° C.	0.36 cu. mm. CO_2 per gm. berry per minute
23.9° C.	1.20 " " " " " " " "

[1] LOREE, as a result of 54 determinations of water content on ripe strawberries of different varieties, found values ranging from 87.9 to 92.8 per cent. In a series of analyses undertaken primarily to show loss of dry weight as a result of respiration, the averages of the two samples showed as a result of the combined effect of water removal and loss of dry weight due to respiration, a loss of 89.78 per cent after 48 hours at 25° C., 91.25 per cent after 72 hours, and 91.56 per cent after 96 hours.

These two rates of respiration are closely similar. They are strikingly different, however, for the rate of respiration of the Missionary variety as previously shown here, the difference being roughly 200 per cent for the rates at about the 10° and 25° temperatures.

In a series of experiments conducted with the Gibson variety, a much higher rate even than that for the Missionary was shown to obtain at the lower temperatures. But the rate for the Gibson at 20° is not much more than half that for the Missionary, and is lower than that for its own 15° rate (table VII).

TABLE VII

RESPIRATION OF GIBSON STRAWBERRY

TEMPERATURE (° C.)	O_2 CONSUMED PER GM. BERRY PER MINUTE (CU. MM.)	NO. OF TRIALS
5	0.7961	6
10	1.4750	14
15	2.7116	19
20	2.5065	13

The point which it is desired to emphasize is that there is an enormous disparity between rates of respiration of different varieties of strawberries. It should be mentioned in this connection that the Missionary strawberries were grown in Florida and the Gibson in Wisconsin. GORE does not state the place of origin of his fruit. The facts that the strawberries of one variety (which respire at a maximum rate at a high temperature) were grown in a region of high temperatures, and those of another variety were grown in a region of low temperatures (and respire most rapidly at comparatively low temperatures) may be a correlation; it is not in any sense a physiological explanation of the difference.

An interesting parallel to this is found in the studies of rates of respiration made upon apples. DRAIN (**11**) states that apple varieties vary greatly in respiration rates, and his conclusions are certainly borne out by his results, the variations being from 300 to 400 per cent, and in some instances from 600 to 800 per cent between respiratory rates of Oldenburg and Maiden Blush apples at the same temperatures. MORSE (**25**) also finds that the Oldenburg respires

twice as much at 25° as the Winesap. DE VILLIERS (32) likewise finds respiration of grapes varying with varietal differences.

These striking contrasts bring into sharp relief the character of the obstacles to an understanding of the nature of respiration. Specific characters of plants are certainly not a guide. It is not the purpose here to present even a summary of the variety of influences which have at one time or another been advanced as "controlling" ones, much less the still larger number of contributing factors. Among the more recent suggestions, however, the one by WILLAMAN and BEAUMONT (33) may be noted. Conclusive data are presented to show that respiration is depressed in an atmosphere of CO_2, but that respiration, as measured by CO_2 output, is renewed with great vigor when the tissues under observation are "aspirated." WILLAMAN and BEAUMONT reject the simplest explanation of this behavior, to wit, that an equilibrium between the CO_2 of the tissues and that of the atmosphere is being reached. They advance the hypothesis that CO_2, by increasing the hydrogen-ion concentration, brings the proteins of the protoplasm to their isoelectric point, thus rendering them more permeable. To the objection that such increase in permeability would operate to increase CO_2 production immediately, they suggest that the increase in permeability would probably be merely potential so long as there was a high content of CO_2 surrounding the tissues, but would become actual as soon as aspiration commenced. No measurement of O_2 consumption is reported in their study.

Numerous attempts have been made to correlate respiratory activity with the presence of catalase. Such a correlation has been found by APPLEMAN (2), SHERMAN (30), STEHLE (31), and others. RHINE (28) found no correlation between respiration and catalase activity in the early stages of seed germination, and thought the connection if any was indirect. The results of three attempts to measure catalase in the strawberry pulp led to the liberation of an amount of O_2 so small as to be difficult of measurement, although strawberry fruits respire actively.

GORE (15) states some of the further perplexities in his study of respiration of fruits: "No satisfactory theory based on the composition or size of fruits has been found to account for the differences in

the respiratory activity . . . . the rate of respiration is not a direct function of content of sugar or of acid and does not depend upon size." (Not correlated with surface area as against mass?)

PALLADIN'S (26) disposition to divide respiration into several different categories, each with its distinct causal agent, as oxidase, nucleo-proteins, protoplasm, etc., and to separate the process into different stages is, it would seem, a helpful concept.

RESPIRATORY RATIO

As a result of about twelve different experiments on as many strawberries, extending over about 60 hours' time, it can be stated definitely that the ratio CO_2/O_2 is greater than one in strawberry respiration. This generalization holds for both green and ripe fruits. The determinations were made by placing the strawberries in the Krajnik apparatus without the NaOH solution to absorb the CO_2 produced. It was necessary to remove the green calyx from the fruits so as to avoid errors as a result of photosynthesis.

It is clear that any changes in the levels of the kerosene under these circumstances will be the result of a difference between the volume of O_2 consumed and the volume of CO_2 released. In all cases the kerosene was depressed in the left side of the tube. The method of measurement, therefore, requires the raising of the mercury to a high point in its tube at the beginning of the experiment, and then restoring the unequal levels of the kerosene columns by lowering it at the end; in short, just the reverse of the manipulation for measuring the oxygen consumption.

Owing to the fact that the movement of kerosene is very much slower than when the entire amount of one of the gases is being measured, the possibility of error is very much greater than in the measurements of O_2 consumption. The value of 1.2 for the respiratory ratio will have to be submitted as an approximation at the present time. The existence of a high respiratory ratio may be associated with production of alcohol and acids, as in the case of anaerobic respiration.

Finally, such a ratio can be interpreted as indicating an oxidation of tannins (CHATIN 8), and more especially of organic acids (GERBER 13).

A decline in acid content seems to be general in the ripening and during the storage of many fruits. For example, MAGNESS (**24**), although he reports a coefficient of respiration of not more than one, finds a consistent decrease in acidity with apples in storage at 32° F.[1] Results of some analyses of the acid content of the strawberry show this to be the case with this fruit. In all cases the results are averages of duplicate samples, and are expressed in number of cc. N/10 NaOH necessary to neutralize the acid content of 1 gm. of strawberry. Samples consisted of from 10 to 35 gm. of fruit.

TABLE VIII

ACID ANALYSES OF STRAWBERRIES

CONDITION	FIRST LOT (CC.)	SECOND LOT (CC.)
Green	2.78	
Partly green		3.02
Early ripe		2.39
Average market condition (ripe)	2.6	
After standing 24 hours at 25°	2.54	
24 hours at 14°		2.03
After standing 48 hours at 25°	2.58	
48 hours at 14°		2.07

From this it appears that a decline takes place in the acidity up to the time that the berries are fully ripe and have reached a stage that may be described as over-ripe.

In the present state of knowledge, there is nothing to prohibit the use of the hypothesis proposed by GERBER that the oxidation of organic acids during respiration supplies an explanation of the high respiratory ratio of the strawberry.

EFFECT OF ETHYLENE GAS ON RESPIRATION

A topic of interest in the current literature in plant physiology is the effect of ethylene gas and other stimulating chemicals in the

[1] The production of "disagreeable flavors" in apples noted by MAGNESS (**24**) as a result of various methods of excluding oxygen, and the "fermented flavor" in cherries and the "very bad flavor" of peaches under similar conditions described by HILL (**18**) are in accord with common experience in the handling of fruits. A point to be emphasized, however, is that a loss of palatability must not be confused with increased acidity.

ripening processes of fruits and vegetables, and concurrently its relation to respiration (CHACE and DENNY **7**, MACK **23**, HARVEY **17**, REGEIMBAL, VACHA, and HARVEY **27**). Does ethylene accelerate the natural process of ripening of bananas, lemons, tomatoes, cantaloupes, etc., with its attendant increase of sugar content and juiciness, or are the heightened colors produced by it due to the occurrence of what may be described as chemical artifacts unrelated to normal maturation?

In an endeavor to secure an answer to this question, several shipments of green Missionary strawberries were ordered from Plant City, Florida, in April (1928) to be sent via air mail and special delivery. The fruit arrived in good fresh condition. Two constant temperature ovens, of a capacity of 102.56 liters, after being carefully sealed with plasticine were used as gas-tight chambers for the experiment, one oven being used for the controls. In one instance about a pint of berries were placed in the oven with ordinary dry air. In a second experiment, the berries in the first instance having shown signs of withering, a number of glasses of water were inclosed with them. In a third trial, two lots of green strawberries were placed in inverted wide-mouthed bottles, the corks of which were protected by tinfoil and penetrated by small pieces of glass tubing, on the lower ends of which were short pieces of rubber tubing clamped by pinch cocks. The temperature was that of the room, about 20° C. Ethylene gas was measured in a gas manometer, and then forced into the oven by a leveling tube filled with mercury. The ethylene was introduced into the bottle in a small section of ordinary glass tubing whose internal capacity had been determined carefully. This glass tube was filled with ethylene by displacement under mercury. It was then connected to the wide-mouthed bottle containing the strawberries, the pinch-cock released, and the gas which it contained allowed to diffuse into the bottle. In the first experiment, doses of 1 part of ethylene to 1000 parts of air were introduced. In the second experiment the dose was cut down to 1 part in 2000. The dosage for the experiment in the bottle was about 1 part in 1500. Ten doses were given in the first experiment, distributed over three days. Eight doses were used in the second experi-

ment with the oven, and also in the third experiment with the bottle, both of which lasted the same length of time.

No results of the effect of ethylene gas could be recognized in any of the experiments. Seen in different lights, spread out, turned over, rearranged, inspected carefully by a number of individuals called to aid, no differences from the controls in redness, yellowness, hardness, juiciness, or taste could be distinguished at all. The direct effect of ethylene on respiration was not measured.

Summary

1. An exceedingly sensitive respirometer is described, by which the rate of respiration of a single strawberry over half a minute of time can easily be measured. The method of operation is explained. Large sources of error inherent in an impracticable method of use of the instrument, but one which plausibly suggests itself, are pointed out.

2. The amount of oxygen used by the Missionary strawberry at temperatures of from 5° to 40° C. is determined. A curve of this respiratory activity is presented.

3. Amounts of oxygen required by strawberries during shipment in carload lots is calculated. Assuming that the oxygen so required is used to oxidize glucose, the amount of heat thereby generated is shown in terms of pounds of ice which it would melt.

4. The maximum initial rate at which strawberries respire is 36.5° C. At the higher temperatures (35°, 36.5°, 37.5°, and 40° C.) respiration quickly falls. As a result, the maximum rate varies with the time over which it is considered.

5. The temperature coefficient for the respiration of the Missionary strawberry is 2.5 for temperatures below 25° C.

6. A temporary increase in the rate of strawberry respiration occurs as a result of exposure to dry air. This rate later declines.

7. Strawberries are very effectively protected from water loss in dry air. The loss is about 2 per cent by weight at the end of 50 hours in dry air, compared with a loss of 1.1 per cent in moist air.

8. Strawberries have a very constant water content, the amount of which is approximately 89.5 to 90 per cent.

9. Deterioration of strawberries in storage at ice-box temperatures as measured by loss in weight is about 4.5 to 5 per cent in two days and 10 per cent in four days.

10. The Gibson strawberry, northern grown, has a much higher rate of respiration at low temperatures than the Missionary strawberry, which is southern grown.

11. Much of the data available upon respiration is inconsistent with the interpretations made of it. This is because of inability so far to reckon in given instances with all the factors affecting respiration.

12. Acid content of strawberries declines as they pass from the green stage to a stage of ripeness just preceding actual deterioration.

13. The respiratory ratio (CO_2/O_2) for ripe fruits is found to be greater than one. An approximate value of 1.2 is given for this ratio. The CO_2/O_2 ratio was determined with the Krajnik apparatus.

14. Ethylene gas in concentrations of 1:1000, 1:1500, and 1:2000 is without effect in hastening the visible changes incident to ripening in strawberries.

The problem here considered was suggested by Professor CHAS. A. SHULL of the Hull Botanical Laboratory of the University of Chicago. To him, and to Dr. SCOTT V. EATON, thanks are due for suggestions and aid in many ways.

STATE TEACHERS COLLEGE
MILLERSVILLE, PA.

[*Accepted for publication January 22, 1929*]

LITERATURE CITED

1. ALLEE, W. C., Studies in animal aggregations: causes and effects of bunching in land isopods. Jour. Exp. Zool. **45**:255–277. 1926.
2. APPLEMAN, C. O., Some observations on catalase. BOT. GAZ. **50**:182–192. 1910.
3. BABCOCK, S. M., Metabolic water; its production and rôle in vital phenomena. Wis. Agric. Exp. Sta. Res. Bull. 22. 1912.
4. BAILEY, C. H., and GURJAR, A. M., Respiration of stored wheat. Jour. Agric. Res. **12**:685–713. 1918.
5. ———, Respiration of cereal plants and grains. II. Respiration of sprouted wheat. Jour. Biol. Chem. **44**:5–7. 1920.

6. BLACKMAN, F. F., Optima and limiting factors. Ann. Botany **19**:281–295. 1905.
7. CHACE, E. M., and DENNY, F. E., Use of ethylene in the coloring of citrus fruits. Jour. Ind. Eng. Chem. **16**:339–340. 1924.
8. CHATIN, A., Études sur la respiration des fruits. Compt. Rend. Acad. Sci. Paris **58**:576–579. 1864.
9. CLAUSEN, H., Beiträge zu Kenntniss der Athmung der Gewächse und des pflanzlichen Stoffwechsels. Landw. Jahrb. **19**:893–930. 1890.
10. CROZIER, J. W., On biological oxidations as function of temperature. Jour. Gen. Physiol. **7**:189–216. 1924.
11. DRAIN, B. D., Temperature and respiratory enzymes of apples. BOT. GAZ. **82**:183–194. 1926.
12. DUVEL, J. W. T., The vitality and germination of seeds. U.S. Dept. Agric. Bur. Pl. Ind. Bull. 58. pp. 96. 1904.
13. GERBER, C., Recherches sur la maturation des fruits charnus. Ann. Sci. Nat. Bot. Ser. 8. **4**:1–280. 1896.
14. GODLEWSKI, E., Über anaerobe Eiweisszersetzung und intramoleculare Atmung in den Pflanzen. Bull. Acad. Sci. Cracovie **8**:623–704. 1896.
15. GORE, H. C., Studies on fruit respiration. U.S. Dept. Agric. Bur. Chem. Bull. 142. 1911.
16. HARRINGTON, G. T., Respiration of apple seeds. Jour. Agric. Res. **23**:117–130. 1923.
17. HARVEY, R. B., Blanching celery. Univ. Minn. Agric. Exp. Sta. Bull. 222. pp. 20. 1925.
18. HILL, G. R. JR., Respiration of fruits and growing plant tissues in certain gases with reference to ventilation and fruit storage. Cornell Agric. Exp. Sta. Bull. 330. 1913.
19. KRAJNIK, B., Über eine Modifikation des Mikrorespirationsapparates. Biochem. Zeitschr. **130**:286–293. 1922.
20. LEITCH, I., Some experiments on the influence of temperature on the rate of growth in *Pisum sativum*. Ann. Botany **30**:25–46. 1916.
21. LOREE, R. E., The nutrient requirements of the strawberry. Mich. Agric. Exp. Sta. Tech. Bull. 70. 1925.
22. LUTHRA, J. C., Some experiments on the effects of dry and moist air on the rate of respiration and breakdown of ripe pears. New Phytol. **23**:131–142. 1924.
23. MACK, W. B., The action of ethylene in accelerating the blanching of celery. Plant Physiol. **2**:103. 1927.
24. MAGNESS, J. R., Physiological studies on apples in storage. Thesis. Univ. of Chicago. 1923.
25. MORSE, F. W., The effect of temperature on the respiration of apples. Jour. Amer. Chem. Soc. **30**:876–881. 1908.

26. PALLADIN, W., Über den verschiedenen Ursprung der während der Atmung der Pflanzen ausgeschiedenen Kohlensäure. Ber. Deutsch. Bot. Ges. 23: 240–247. 1905.
27. REGEIMBAL, L. O., VACHA, G. A., and HARVEY, R. B., The effect of ethylene on the respiration of bananas during ripening. Plant Physiol. 2:357–359. 1927.
28. RHINE, LOUISA E., Divergence of catalase and respiration in germination. BOT. GAZ. 78:46–67. 1924.
29. KOLKWITZ, R., Über die Athmung ruhender Samen. Ber. Deutsch. Bot. Ges. 19:285–287. 1901.
30. SHERMAN, HOPE, Respiration of dormant seeds. BOT. GAZ. 72:1–30. 1921.
31. STEHLE, R. L., and MCCARTY, A. C., Further data concerning the alleged relation of catalase to animal oxidations. Jour. Biol. Chem. 42:269–272. 1927.
32. DE VILLIERS, F. J., Physiological studies of the grape. Union So. Africa Dept. Agric. Sci. Bull. 45. 1926.
33. WILLAMAN, J. J., and BEAUMONT, J. H., The effect of accumulated carbon dioxide on plant respiration. Plant Physiol. 3:45–59. 1928.

SPECIALIZATION IN SECONDARY XYLEM OF DICOTYLEDONS

I. ORIGIN OF VESSEL

FREDERICK H. FROST

(WITH TWENTY FIGURES)

Introduction

So relatively few existing angiosperms have been studied by morphologists and anatomists that adequate data are not available, at the present time, for constructing a comprehensive systematic classification of woods. It is true that more or less serviceable keys for the identification of commercial woods of certain specific regions have been formulated, but such keys cannot be relied upon in identifying woods of other regions, nor in identifying specimens from non-commercial species. Although the ultimate solution of the problem of classifying and identifying woods is dependent upon the compilation of larger volumes of reliable descriptive data, the investigation of the major trends of evolutionary specialization in the secondary xylem should be of considerable significance in arranging and interpreting these data and in visualizing the salient features of a natural classification. For example, if the evolutionary specializations of the flower and of the stem are closely correlated, the classification of the woods may well be made to parallel that based upon floral structures. On the contrary, if there is relatively little correlation in the evolutionary specialization of these organs,[1] an independent system of classification may have to be formulated for woods. A knowledge of the trends of specialization is also an aid in distinguishing between environmental variations and hereditary sequences in the individual, and makes it possible to refer specific hereditary variations to a definite scale, defined by the wide variation of evolutionary development.

[1] There is much evidence, both observational and theoretical, to indicate that such is the case. While the agreement between wood and floral structure in a genus is close in many cases, the degree of correlation becomes lower as the size of the classification unit is increased.

The writer plans to trace the major lines of specialization in the secondary xylem and to publish his results in a series of related papers. The present paper, the first of this series, discusses the origin of the vessel and the vessel segment; the second paper will deal with the specialization of the end wall of the vessel segment; the third with the changes which the lateral pitting of vessel segments undergo during evolutionary development; and the fourth with the form and distribution of vessel segments, etc. A list of new diagnostic characters and a discussion of their use in identification will be given in the final paper of the series.

Methods

Four methods have been employed in studying the evolutionary development of the characteristics of wood. Although these methods are not new there has been so much confusion in their application that it seems desirable to restate them in detail.

The first method is the method of association, and may be described as follows. If it is possible to determine which of two characters is primitive, and if it be assumed that the two structures are genetically related in a direct line, then it follows that the primitive condition of the advanced structure will be similar to the general condition of the primitive structure. If it is found in the application of this method that such similarity does not exist to a considerable extent, it follows that the assumption of direct genetic relationship is incorrect, or that the two structures are so widely separated in the evolutionary scale that the primitive condition of the advanced structure is lost. If the similarity is close, however, both in the homologous and analogous sense, it follows that the assumption is correct, and it becomes easy to determine statistically the primitive condition of the advanced structure. This method is of use only when the characteristic in question is variable and when it is possible to obtain statistically sound samples from comparable regions.

The second method is as definite as the first and involves no complex assumptions. This is the method of correlation, and it assumes that in a given homogeneous tissue, such as the secondary xylem, there will be definite correlations, in the strict statistical sense, between the degrees of specialization of the chief characteris-

tics in a large random sample. That is, the rates of evolutionary development will be correlated; hence it follows that those characteristics which correlate with a primitive character, established as primitive by the method of association, will themselves be primitive, and those that correlate with an established specialized character will be specialized. This does not mean, of course, that a specialized and a primitive character cannot be found in the same tissue of a given species, since correlations are expressions of major trends or average relationships.

A correlation, unless it is perfect, is a description of an average or major trend. This implies that there are exceptions to the general rule if the population is studied from the individual rather than the group standpoint. This consideration leads directly to the third method, the method of exceptions. It is the study of the individual exceptions from the average line of specialization, when dealing with the development of specific characters, which will throw light upon special or unusual variations from the main course. The exceptions do not, of course, exist until the rule is established, and for this reason the method of exceptions is a corollary of the method of correlation. Both methods give evidence which may be directly interpreted: the latter the major lines of evolutionary development and the former the minor lines.

The statistical method is essentially a method for the study of the characteristics of a large group by taking individual samples, at random, from the group. Experience, in agreement with theory, has repeatedly shown that the accuracy of conclusions drawn from statistical results is proportional to the number of cases used in deriving the statistical constants. For this reason the number of individual woods measured and studied in these investigations is large in order to insure a fair representation of dicotyledonous woods in general.[2] Where the numbers were small, due to lack of material,

[2] The writer is fortunate in having access to the large collection of microscopic slides of woody plants which has been brought together and prepared under the direction of Professor I. W. BAILEY. This collection is representative of the woods of the world, and includes 63 of the 76 orders and 137 of the 264 families listed by HUTCHINSON. There are well over 800 genera and 2000 species in the collection. The writer has also drawn material from his own collection of the woods of the United States which at present includes some 150 genera and 450 species.

the results were safeguarded by statistical checks. The writer feels that investigators in this field have too often drawn rather sweeping conclusions from insufficient material.

THOMPSON (19) gives a detailed discussion of correlated specialization, his method of Cartesian co-ordinates in particular giving a graphic proof of the validity of the concept.

The fourth method of studying evolutionary trends is the method of sequences, and it is essentially concerned with the reconstruction of evolutionary variability from the observable variation of living forms. Variation in living forms may be ontogenetic, positional, irregularly disposed in a tissue, or may be found among the individual units of a taxonomic group. There is no fundamental distinction between the various types since all types are caused by differential rates of specialization and the persistence of primitive features.

GEGENBAUR (11), MORGAN (17), SCOTT (18), and others have shown that any particular sequence, regardless of type, may be cenogenetic, and that the direction of the specialization cannot be determined safely from a consideration of the sequence alone. For these reasons the attack on a given problem resolves itself into first determining by the methods of association and correlation which characters are primitive and which are specialized, and then constructing the intervening stages by an application of the method of sequences. When used in this manner the method is not subject to the criticisms which have been made against the doctrines of recapitulation, reversion, and conservative regions, since it is possible to distinguish between cenogenetic and palingenetic variations, and since the direction of the specialization is determined by independent methods.

The theories in regard to the origin of the vessel in the dicotyledons are various, and the literature is complicated, confused, and contradictory. No critical historical account is given at this stage of the presentation of the problem; on the contrary, it seems more logical to reattack it from the beginning by an application of the methods outlined, and then, with the knowledge which a broad survey of the secondary xylem of the dicotyledons gives, to enter into a detailed historical discussion.

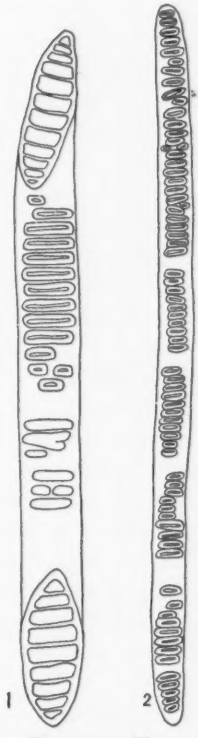
Initial considerations

The term vessel was concisely defined by VON MOHL (16) as follows:

> If this remains closed (the end wall) after its development is completed it is called a cell, *cellula*, but if a row of utricles arranged in a line becomes combined, during development, into a tube with an uninterrupted cavity, through the absorption of the cross walls, a compound elementary organ is produced, the *vessel*.

While this definition was accepted and widely used by many leading investigators of the nineteenth century, more recently the term vessel has been frequently misapplied. A vessel is clearly by, VON MOHL's definition, a series of individual cells, properly called vessel elements or vessel segments, which are joined end to end with perforate division walls.

A vessel segment (fig. 1) is chiefly distinguished from a tracheid (fig. 2) by the presence of an end wall, specifically distinct from the other walls, and by the presence of a perforation. The first point has been little emphasized in discussions concerning the origin of the vessel segment; its importance will be made clear in the discussion which follows. There is little fundamental difference in regard to the pitting of the vessel and the tracheid; both may have the typical elongate scalariform type of pit, the transitional type, or the opposite or alternate arrangement of the very common circular type.



FIGS. 1, 2.—Fig. 1, diagram of typical vessel segment with scalariform perforations and scalariform lateral pitting; fig. 2, *Trochodendron aralioides*, typical scalariform tracheid from secondary xylem.

Discussion

It is of primary importance, in determining the manner in which the vessel segment originated, to establish which of the various types

of vessel segments is primitive. This can be accomplished by an application of the methods of association and correlations if we grant that the following assumptions are essentially correct: (1) that the theory of evolution is correct; (2) that specialization results in a series of graded forms; (3) that the tracheid is a more primitive structure than the vessel segment; and (4) that the vessel segment is genetically related to and derived from the tracheid. The correctness of the first and second assumptions is almost beyond doubt; the third is supported directly by evidence from the fossil record and by considerable collateral evidence; the fourth is universally held to be true since tracheids and vessel segments serve the same function, are in the same positional arrangement, and are morphologically similar. We need not accept these assumptions as facts, however, since if they are incorrect there will be a decided lack of coherence in the data. If, however, it can be shown that the many facts which it is possible to assemble become unified under these assumptions it further establishes their correctness.

If these assumptions are considered to be true, it follows, by an application of the method of association, that the truly primitive vessel segment will be characterized by the features which define tracheids in general. It must be remembered in this connection, however, that secondary vessel segments must be compared with secondary tracheids; that comparable regions of the plants must be used for comparison. What, then, are the characteristics which tracheids, as a whole, possess which should be incorporated in the organization of the primitive vessel segment? In brief there are six features: (1) great length, (2) small cross-sectional area, (3) angularity of outline, (4) thin walls, (5) constant width of walls in transverse section, and (6) the absence or very slight development of an end wall.

1. GREAT LENGTH.—Tracheids are much longer than vessel segments. Table I gives the averages computed from measurements given by BAILEY and TUPPER (3). These figures represent, as a rule, lengths of vessel segments and tracheids taken from mature secondary wood, and the grand averages are computed from averages of each species.

It follows, in view of the great differences in length of tracheids

and vessel segments, that primitive vessel segments will be long, and by the method of correlation, that the characteristics of long vessel segments will be primitive. BAILEY and TUPPER (3 table II) indicate the type of perforations possessed by long (primitive) vessel segments.

In some 270 odd species from 114 families there are seventeen species listed whose vessel segments average more than 1.2 mm., and all have exclusively scalariform perforations. In other words, the correlation between very long vessel segments and scalariform end walls is positive and perfect, or, in statistical terms, the correlation

TABLE I

GROUP	NO. OF SPECIES	TRACHEID LENGTH (MM.)	VESSEL SEGMENT LENGTH (MM.)
Cordaitales	2	5.05	.....
Bennettitales	2	5.25	.....
Cycadales	1	0.80	.....
Ginkgoales	1	3.50	.....
Coniferales	146	3.64	.....
Gnetales	3	.....	0.96
Dicotyledons	276	.....	0.57

coefficient is -1. Again, of the forty-six species whose vessel segments average 1 mm. or more, thirty-two have entirely scalariform perforations, six have scalariform-porous perforations, and eight have oblique porous perforations with vestiges of the scalariform condition. Not one of these species has vessel segments with the typical transverse porous end wall. For contrast, at the lower end of the scale there is a perfect correlation between transverse porous perforations and shortness. In the thirty-two species whose vessel segments average 0.02 mm. or less, all have transverse porous perforations. An analysis of the correlation in the 274 species is shown in table II.

In this particular case the evidence is overwhelmingly in favor of the scalariform type of perforation being the most primitive.

2. SMALL CROSS-SECTIONAL AREA.—Since on the average tracheids are much smaller in cross-sectional diameter than vessels, it follows, by the method of association, that the primitive vessels

will be small,[3] and by the method of correlation that the characteristics of small vessels will be primitive. Tracheids in general average about 0.03 mm. in diameter. Forty dicotyledonous species with scalariform perforations, picked at random from the collection, average 0.067 mm. in diameter, whereas forty diffuse porous woods, with transverse porous perforations, average 0.12 mm. In other words, the long vessel segments with exclusively scalariform end walls are also small in diameter similar to tracheids, while the short vessel segments with transverse porous perforations are large in diameter dissimilar to tracheids.

TABLE II

ANALYSIS OF SPECIES

PERFORATIONS	VESSEL SEGMENT LENGTH				
	1.3–2.0 mm.	1.0–1.3 mm.	0.6–1.0 mm.	0.3–0.6 mm.	0.0–0.3 mm.
Scalariform	17*	15	17	5	0
Scalariform-porous	0	6	7	5	0
Porous-oblique, vestigial scalariform	0	8	12	14	0
Transverse-porous	0	0	30	106	32

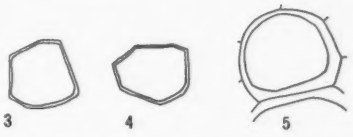
* In almost all cases only one species is taken from each genus. Since the species of a genus are usually alike as regards wood structure, it would give improper weight to certain types to increase the number of cases by taking more than one representative from each genus.

It will be noted that only diffuse porous woods were used in making these measurements, to afford a better comparison with the purely scalariform woods which are necessarily diffuse porous. Ring porous woods tend to have larger vessels than diffuse porous woods. There is a high correlation between the diffuse porous condition and the presence of scalariform perforations. As one might expect, the distribution of primitive vessels follows the distribution of tracheids (that is, is diffuse, or more or less evenly spaced throughout the xylem). These data indicate that the scalariform perforation is primitive, a result in complete harmony with the conclusions obtained from the length data.

3. ANGULARITY OF OUTLINE.—We should also expect that the primitive vessel segment would be angular in transverse outline since tracheids in general are angular. The forty scalariform per-

[3] BROWN (8) has recently reached this same conclusion.

forate species studied in regard to cross-sectional diameter must therefore be angular if the previous conclusions are correct. As a matter of fact all are angular in outline, or the correlation between the two primitive characteristics, angularity and small diameter, is perfect in these species. Of the forty porous perforate species, six are angular while thirty-four are round or oval. The correlation between great length and angularity is also perfect since the seventeen species with vessel segment lengths averaging over 1.2 mm. have angular vessels in all cases. Fig. 3 illustrates an angular tracheid from *Sequoia sempervirens*, and fig. 4 an angular vessel from *Gordonia lasianthus*. This is further proof that the scalariform perforation is a primitive feature in the wood of the dicotyledons.



FIGS. 3–5.—Fig. 3, *Sequoia sempervirens*, angular tracheid in transverse section; fig. 4, *Gordonia lasianthus*, angular vessel in transverse section; fig. 5, *Exothea paniculata*, common type of vessel with unevenly thickened walls.

4. THIN WALLS.—In nearly all cases tracheids possess evenly thickened walls, and if the forty supposedly primitive woods are truly primitive they must also have vessels with evenly thickened walls. That this is true is shown by the following figures. Of the forty woods with scalariform perforations, thirty-nine have evenly thickened walls, in contrast to the forty porous perforate species in which only nine have evenly thickened walls. Fig. 5 illustrates the unevenly thickened vessel type from *Exothea paniculata*, which may be compared with the evenly thickened type shown in fig. 4.

5. CONSTANT WALL WIDTH.—Tracheids are characterized by thin walls, and in this case all of the forty scalariform species are thin-walled. In the porous group ten have thin walls, thirteen have vessels which are thick-walled when in contact with other vessels, and seventeen have thick walls only.

6. UNDEVELOPED END WALL.—In the scalariform types the end wall is highly inclined or absent, and does not materially change the characteristic tracheid-like shape of these primitive vessel segments. In the porous species the end wall is usually transverse, and greatly

alters the shape of the vessel segment from the original tracheid-like form.

In summary, the vessel segments which retain the tracheid characteristics of length, small diameter, angularity of outline, evenly thickened walls, thin walls, and an unspecialized end wall are found, as they should be if all these characteristics are primitive, in the same group of plants, and are dominantly characterized by the presence of scalariform perforations similar to those found in *Pteris* and totally different from the Gnetalian type. The conclusion is irresistible that the scalariform perforation is the most primitive type found in the dicotyledons.

Now that the nature of the primitive perforation has been determined, its mode of origin from the tracheid may be discussed. Since there is a close agreement between the morphology of certain vessel segments and tracheids in general, indicating that this type of vessel segment must be primitive, it is logical to infer that there will be a marked agreement between the pitting of these vessel segments and the pitting of the tracheid type from which they were evolved by evolutionary specialization. It is not known, of course, which type of pitting characterized these tracheids, since the pitting of tracheids is variable, but we can discover what type dominates in the primitive vessel segments, and we may conclude that this type will be similar to that from which it was produced. This is a reverse application of the method of association.

Of the seventeen species previously referred to, with very long vessel segments, fourteen are characterized by scalariform lateral pitting and three by transitional to opposite pitting. This is indeed meager evidence, due to the small number of cases, but it is a decided indication of correlation. BAILEY and TUPPER have supplied abundant evidence, however, which is summarized in table III.

In group 1 the scalariform lateral pitting is dominant, in group 2 transitional and opposite pitting dominates, in group 3 opposite and alternate pitting dominates, and in group 4 alternate pitting is most common.

The writer's observations, based upon 104 scalariform, scalariform-porous, and vestigial scalariform woods, gives the results shown in table IV.

Tables III and IV show that there is a high correlation between the primitive scalariform condition of the end walls and the scalariform condition of the side walls. It is rather interesting to note

TABLE III

ANGIOSPERMAE—DICOTYLEDONEAE
(BASED ON 274 SPECIES IN 114 FAMILIES)

PERFORATIONS	TYPES OF LATERAL PITTING: LATERAL PITTING	PERCENTAGE
(1) Highly inclined, entirely scalariform	Scalariform and opposite	86
	Opposite and alternate or alternate	14
(2) Inclined, scalariform, porous	Scalariform and opposite	80
	Opposite and alternate or alternate	20
(3) Inclined porous, vestigial scalariform	Scalariform and opposite	11
	Opposite and alternate or alternate	89
(4) Transverse porous	Scalariform and opposite	6
	Opposite and alternate or alternate	94

that in group 1 of the table given by BAILEY and TUPPER, 83 per cent of the species have other tracheary cells with distinct bordered pits, whereas in group 4 only 17 per cent of the species have distinct bordered pits in their fibrous elements. The scalariform condition of

TABLE IV

PERFORATIONS	TYPES OF LATERAL PITTING: LATERAL PITTING	PERCENTAGE
(1) Highly inclined, entirely scalariform	Scalariform	57
	Opposite	33
	Alternate	10
(2, 3) Inclined scalariform-porous or vestigial scalariform	Scalariform	19
	Opposite	27
	Alternate	54

The transitional type of pitting is classed as scalariform or opposite, depending upon which it most nearly resembles.

both the end and the side walls therefore correlates with the accepted primitive type of fiber pitting.

The only interpretation which can be given these data is that scalariform lateral pitting is primitive in the organization of the

vessel segments of the dicotyledons. There is, then, good reason to suppose that the tracheid type from which these primitive vessel segments were derived was characterized by scalariform lateral pitting. In other words, the vessel segment originated in the dicotyledons in precisely the same manner as it originated in *Pteris*, by the loss of membranes from the scalariform pits at each end of the tracheid and the formation of an end wall.

If this reasoning and interpretation are correct, one should expect to find, in the secondary xylem of primitive dicotyledons, transitions from scalariform tracheids to scalariform vessel segments, which would record the evolutionary change. An examination of a great number of woods failed to reveal any evidence that such transitions occur in the secondary xylem, or that true scalariform tracheids are present in the secondary wood of forms which possess vessels. This can mean either that the theory is entirely incorrect or that the secondary xylem is so highly specialized that such transitions have been lost. The writer's attention next centered on the primary xylem, and it was at once clear that, in species which possess a primitive secondary xylem, such transitions not only occur but are also in positional sequence from the pith outward, and show, in great detail, the transition from the scalariform tracheid to the scalariform vessel segment. If this is a true evolutionary sequence, there must be some reason why it occurs in the primary and not in the secondary wood, and why the transition is in positional sequence.

Measurements and examinations were accordingly made of the primary wood of a great number of species. The results are summarized in table V.

It will be noted that the dicotyledons readily segregate into three major groups, and that group 2 is composed of species with primitive scalariform perforations in the primary xylem, and specialized porous perforations in the secondary xylem. This means that in this group the secondary xylem is more highly specialized than the primary. In the first protoxylem group vessels are frequently absent, a fact which further substantiates this opinion.

In the protoxylem, metaxylem, and secondary xylem of the first annual ring, the vessel segments decrease in length in groups

1 to 3, as the end wall becomes more highly specialized. These correlations correspond exactly with the data, previously given, for the mature secondary xylem. There is, then, striking evidence to show that the evolution of the vessel proceeded along the same lines in these three regions.

In all three classes there is also a reduction in average length from the protoxylem, to the metaxylem, to the secondary xylem when each region is considered as a unit. Since length is a primitive characteristic, it may be concluded that the vessel segments of the protoxylem are less specialized than those of the metaxylem, and

TABLE V

TRACHEID AND VESSEL SEGMENT LENGTHS*

PERFORATIONS	PROTOXYLEM (MM.)	METAXYLEM (MM.)	FIRST FORMED SECONDARY XYLEM (MM.)
(1) Absent or scalariform in primary xylem, scalariform in secondary xylem	1.948	1.647	0.709
(2) Scalariform in primary xylem, porous in secondary xylem	1.331	1.203	0.426
(3) Primary porous, secondary porous	0.974	0.751	0.338

* These figures are average lengths of scalariform tracheids, when vessels are absent, or of vessel segments.

that those of the metaxylem are less specialized than the vessel segments of the secondary xylem. It will be noted that the greatest average lengths occur in the protoxylem and metaxylem of group 1, and it was in species of this class that the tracheid to vessel segment transitions were found.

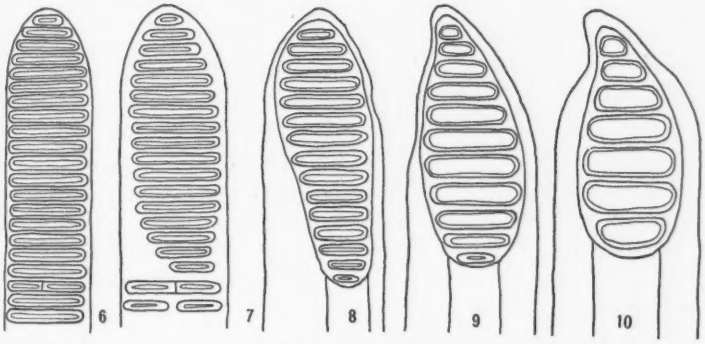
While the measurements in this instance apply to the protoxylem and the metaxylem as a unit, only the first formed secondary xylem was considered. In any given angiosperm, as a rule, there is a tendency for the cambial initial to increase in length with increasing age. This increase in length causes an increase in the length of the corresponding vessel segments, since, as BAILEY (1) has shown, there is a close correlation between vessel segment and cambial initial length. While the increase is not great, it tends to raise the length average of the secondary xylem as a whole, and to make the differences between the means of the secondary xylem and the metaxylem more comparable with the differences between the means of the

protoxylem and metaxylem. While there is no doubt that the vessels of the secondary xylem are more highly specialized than the vessels of the primary xylem, it would seem fair to argue that the vessels of the mature wood are less specialized than the vessels of the early secondary xylem, since the vessel segment length is longer in the former region. While this argument superficially appears to be sound, a consideration of the ontogenetic variations in primitive plants in general shows it to be false. For example: vessel segment length averages about 1 mm. in the mature wood of scalariform perforate species, and this is more tracheid-like than the young wood with an average vessel segment length of only 0.7 mm., but when this mature wood average is compared with the average tracheid length of the mature wood of the gymnosperms, it is no more tracheid-like than the young wood average, since the tracheids of the gymnosperms also increase in length with increasing age of the tree. Relatively the comparison is closer between the young regions, since the tracheids of the gymnosperms increase to a greater extent with age than the vessel segments of the angiosperms. The increase in length which the cambial initial undergoes during its life history is, evidently, a general phenomenon which is correlated with the arboreal habit and which should be rendered constant when comparing average lengths. This can be accomplished by treating the secondary xylem as a unit, or by comparing young wood with young wood and old wood with old wood.

The data presented in table V indicate that the reason no transitions from the scalariform tracheid to the scalariform vessel segment were found in the secondary xylem is that the vessel segments in this tissue are so highly specialized that such transitions have been lost. The reason the transition is still present in the primary wood is explained by the very strong probability that the primary wood is much less specialized, in respect to the type of vessel segment, than the secondary wood, a supposition which agrees with all the facts.

Before proceeding with a discussion of the details of this transition, it is possible further to verify this hypothesis. If the interpretation of the data of table V is correct, this protoxylem to secondary xylem transition should reflect the accepted sequence from

scalariform to porous perforations; that is, if the reasoning holds for one case it should hold for another. As a matter of fact, an inspection of almost any plant with scalariform perforations in the primary wood and porous perforations in the secondary wood will show this transition in considerable detail. The details of this line of specialization will be covered in the next paper of this series; it is only necessary at this time to show that there is complete harmony in the evidence. This sequence of tracheid to vessel segment transition is shown in the following genera: *Ilex*, *Nyssa*, *Viburnum*, *Cornus*, *Rhododendron*, *Schizandra*, *Gordonia*, *Symphlocos*, and many others.



FIGS. 6–10.—*Schizandra chinensis*, transitional stages showing origin of vessel from scalariform tracheid.

In *Schizandra chinensis* the transition is especially striking, since the vessel segments of the secondary wood have a rather highly specialized type of scalariform perforation, quite distinct from the primitive type found in the metaxylem and late protoxylem. The spiral protoxylem elements are tracheids since they do not possess an end wall or a perforation. The region between the protoxylem and metaxylem is characterized by long tracheids whose lateral walls are marked by distinct scalariform pits. A tracheid of this type is illustrated in fig. 6, and it will be seen that no end wall has developed. There is no difference in the pitting of these pre-vessel types of tracheids and the scalariform lateral pitting of vessel segments. The pitting at the middle is the same as the pitting at the

ends, and there is no indication of the loss of pit membranes. Fig. 7 represents the next type of cell in this positional and evolutionary sequence. That this cell is neither a typical tracheid nor a typical vessel segment is evident. No end wall has as yet developed, but the scalariform pitting at the ends of the cell has taken the form of a perforation, while the lateral pitting is breaking up into the transitional condition. These cells are long, their diameter is small, their walls are thin and evenly thickened, their outline angular, and the end wall is unspecialized, characteristics which are common to both tracheids and primitive vessel segments.

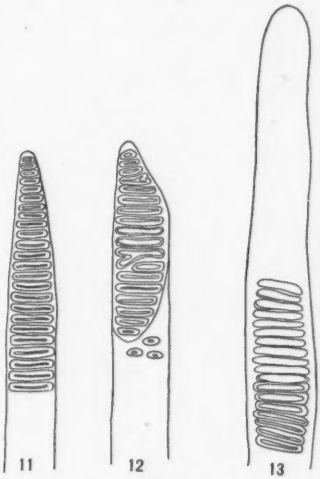
Fig. 8, taken from the middle of the metaxylem, illustrates the next step in this series. Here the perforation has been formed by the loss of the pit membranes from the scalariform pits at the end of the cell resulting in a vessel segment, which still, in general, retains tracheid-like characteristics. Fig. 9, from the end of the metaxylem, shows the further changes which occur at the end of the cell: the increase in diameter, the establishment of the end wall, the reduction in the number of bars of the perforation, and the widening of the apertures resulting in a vessel segment, much more vessel-like than tracheid-like. These cells are, of course, much shorter than those illustrated in figs. 6 and 7. Fig. 10 represents the end product of the series, the typical vessel segment of the secondary wood of *Schizandra chinensis*. The original scalariform lateral pitting has been replaced at this stage by the transitional to opposite pitting characteristic of the secondary wood. Vestigial perforations, as one would expect, are found occasionally in the secondary wood. These are similar to those illustrated in fig. 8, but the scalariform tracheids have entirely disappeared.

Figs. 11 and 12 show the origin of the vessel as reflected in *Nyssa aquatica*. The contrast between the scalariform tracheid and the end product is not so striking in this case because the vessel segments of the secondary xylem are not so highly specialized as they are in *Schizandra*.

In view of the proof offered that the transition from the protoxylem to the secondary xylem represents a primitive to specialized sequence which is palingenetic, and the accurate portrayal this sequence gives in the change from scalariform to porous perforation,

there is little reason to doubt that the detailed transitions here illustrated give a connected picture of the evolution of the scalariform vessel segment from the scalariform tracheid.

In primitive woods which show the tracheid to vessel segment transition, the elements which are intermediate between tracheids and vessels generally show no sharp transition from the scalariform condition of the end wall to the scalariform condition of the side wall. It follows that this arrangement should occasionally be found in the secondary vessel segments of species with primitive wood structure, and the point is clearly illustrated in fig. 13 from *Laurelia novae-zelandii*, where this condition is somewhat common in the secondary wood. This is a very primitive arrangement, of course, and therefore there should be a correlation between this characteristic and great length. To test this, the species with exclusively scalariform perforations were divided into two groups: (1) those with a gradual transition in the type of pitting from the end to the lateral wall of the vessel segment; and (2) those with a sharp transition in the type of pitting. The lengths of the vessel segments in these two groups were then measured and the averages are given in table VI.



FIGS. 11–13.—Figs. 11, 12, *Nyssa aquatica*, transition showing development of scalariform perforation from scalariform tracheid; fig. 13, *Laurelia novae-zelandiae*, true palingenetic sequence from scalariform lateral pitting to scalariform openings.

In the first group the shortest vessel segment is 0.7 mm., and twenty-two species have vessel segments which average 1 mm. or more. In the second group the shortest average vessel segment length is 0.4 mm., and only eight species average 1 mm. or more (length data taken from table II of BAILEY and TUPPER). It will be seen that this primitive characteristic, which is really a combina-

tion of two primitive characters, is closely correlated, as one would expect, with great length.

An interesting point in this connection was made by BROWN (7), who argued that the rate of specialization of the end wall would be slower than the rate of change of the pitting of the side walls, because the end walls would be in contact with vessel segments only, whereas the side walls would be in contact with a variety of cell types. While BROWN'S point is well made, as evidence to be presented in a later paper will show, it must be remembered that there

TABLE VI

TYPE	NO. OF SPECIES	AVERAGE LENGTH (MM.)*
1.....	29	1.27
2.....	22	0.87

* Average length of vessel segments with scalariform perforations is 1.09 mm. Average length of 1.27 mm. of vessel segments with both scalariform side and end walls really represents a multiple correlation, since it is the mean of the prediction of length from two factors. That it is greater than the prediction of length from scalariform ends alone is to be expected both statistically and logically.

are a number of species with primitive scalariform pitting on both end and side walls, and that the difference in rate of change, while distinct, is not of great magnitude and does not have much effect on the correlation between side and end wall characteristics.

So far no mention has been made of the magnoliaceous genera *Trochodendron*, *Tetracentron*, and *Drimys*, in which vessels are absent in the wood. No discussion of the origin of the vessel would be complete without a consideration of these very interesting and unusual forms.

Trochodendron is found in Japan and Formosa and *Tetracentron* in central and western China. The individuals of these monotypic genera reach a considerable size and are found in moist montane habitats. *Drimys* is rather widely distributed in the southern hemisphere and is found as a small tree or shrub growing under somewhat mesophytic conditions.

All three genera have a primary and secondary xylem which is composed entirely of tracheids and parenchyma. There is no indication of the formation of perforations or end walls. The wood of these

genera is unquestionably very primitive, and represents an offshoot from that group of plants which gave rise to the angiospermous type of wood by the acquisition of vessels. The type of tracheid which they possess should therefore be quite similar to the type from which the dicotyledonous vessel segment was derived. A study of the histology of their tracheids should therefore give an independent check on this theory of the origin of the vessel.

Trochodendron has mature secondary tracheids which average 4.5 mm. in length; *Drimys* has secondary tracheids which average 4.3 mm. in length; and in *Tetracentron* the average length is 1.8 mm. in the first annual ring, which indicates that the mature wood average is well over 3 mm. These averages are more than twice as long as any observed in primitive dicotyledons with vessels, and these facts substantiate the opinion that great length is a primitive characteristic, and that the vessel segment was derived from a long tracheid.

Since it has been found that scalariform lateral pitting is primitive, and that the type of tracheid which gave rise to the vessel segment was characterized by scalariform pitting, it would seem to follow that the tracheids of these genera should have scalariform lateral pits. The tracheids of the primary wood, with the exception of the spiral protoxylem elements, are scalariform and longer than the first formed secondary tracheids. The tracheids of the secondary xylem have either scalariform or circular pits; in *Tetracentron* and *Trochodendron* the former condition is common in the spring wood and the latter in the summer wood (figs. 2, 14); in *Drimys* the circular type of pitting predominates in the older secondary xylem. In these genera, as in the primitive dicotyledons with vessels, the primary wood specializes more slowly than the secondary xylem, and it is therefore quite apparent that the scalariform type of lateral pitting is more primitive than the circular type. The only difference between the scalariform tracheids of the primary wood of these species and the scalariform tracheids found in the primary wood of the primitive dicotyledons with vessels is that of length.

In conclusion, while these genera may well represent a blind line of specialization, there is every indication that they still retain many of the characteristics of the plants which gave rise to the dicotyle-

dons as we now know them, and that the scalariform tracheids of their primary wood are very similar to the type which gave rise to the vessel segment and thereby the vessel. The writer feels secure in venturing the prediction that angiospermous fossil wood will eventually be found which will show, in the secondary xylem, all the stages in the formation of vessel segments from long scalariform tracheids.

In the preceding pages an attempt has been made to bring together all the available evidence bearing on the origin of the vessel,

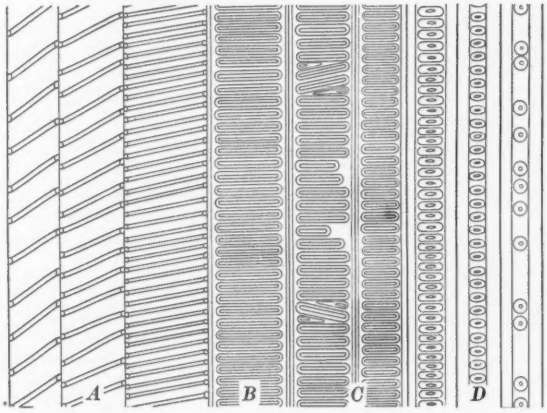


FIG. 14.—*Trochodendron aralioides:* (*a*) spiral protoxylem elements; (*b*) scalariform tracheid from metaxylem; (*c*) scalariform tracheids from spring wood of secondary xylem; (*d*) circular pitted tracheids from summer wood of secondary xylem. Spring wood tracheids of each annual ring are scalariform, while those found in summer wood are marked with circular pits.

and to evaluate this evidence in accordance with the principles outlined in the introduction.

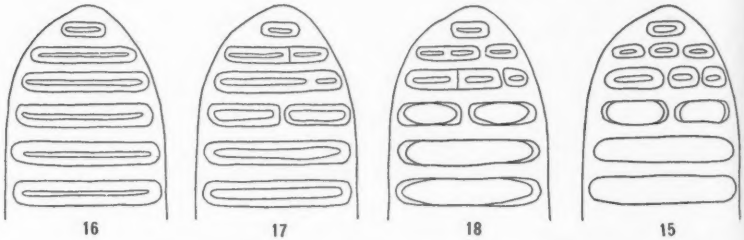
It has been shown that the six features which are common to tracheids in general are all incorporated in the organization of vessel segments with scalariform perforations, and that these vessels also have scalariform lateral pitting. These facts led to the conclusion that the scalariform condition of the end and side wall is a primitive characteristic, and that the tracheid type which gave rise to the vessel segment had scalariform lateral pitting. It was then found

that certain genera, which are primitive in these respects, possessed sequences which showed in detail the stages in the evolution of the vessel segment from the scalariform tracheid. The histology of the tracheids of the primitive vessel-less genera of the Magnoliales indicates independently that these conclusions are correct. These facts are the main points in the argument that the scalariform perforation developed from scalariform lateral pitting by the loss of pit membranes from the ends of the tracheid, and form, with many minor facts, a unified whole when considered in the light of this theory. Why then, if the story of the origin of the vessel has been so clearly written in the secondary and primary xylem of existing dicotyledons, has there been such diversity of opinion in regard to this fundamental point? The writer will endeavor to illustrate in the following historical discussion that the lack of agreement is not due to the existence of contradictory facts, but is a result of the general use of methods which do not give reliable results when used independently of the methods of association and correlation.

Historical discussion

Although DE BARY (9), as early as 1884, suggested that the scalariform perforation is a modification of the end of a scalariform tracheid by the loss of the pit membranes, the first detailed theory of the origin of the vessel was described by BOODLE and WORSDELL (6) for the Gnetales and certain representatives of the dicotyledons. They considered, in the case of the dicotyledons, that the perforation developed by the fusion of circular pits into scalariform pits, which in turn lost their membranes and became scalariform openings. The theory was based upon transitions from circular pits to scalariform pits to scalariform openings, which they found and illustrated in several distinct cases. While such transitions occur, it is difficult to find any justification for interpreting the direction of the specialization as from circular pits to scalariform openings, rather than from scalariform pits to circular pits, or for assuming that the sequence is truly palingenetic rather than cenogenetic. Fig. 15 illustrates a sequence of this nature which in the light of present knowledge is rather easy to interpret. While it is known that the scalariform opening is derived directly from the scalariform pit, the data

in tables III and IV show that the scalariform and not the circular pit is primitive. Table VI also shows that the vessel segments with both scalariform lateral pitting and scalariform perforations are decidedly more primitive than vessel segments with circular lateral pitting and scalariform perforations. Figs. 16–18 illustrate diagrammatically the course of events which lead to the condition shown in fig. 15. Fig. 16 shows the now familiar scalariform tracheid which is to give rise to the vessel segment. In this case the three upper scalariform pits do not lose their membranes during the development and specialization of the end wall, but specialize as



FIGS. 15–18.—Diagrammatic illustration of formation of cenogenetic sequence

pits. This specialization results in a change from the primitive scalariform arrangement to the advanced opposite circular one. Figs. 17 and 18 show how this process occurs. The lower three scalariform pits of fig. 15 lose their membranes and specialize as scalariform openings; a specialization which results, as will be shown in detail in the next paper of this series, in the complete loss of the borders around the openings but with only a slight change in their scalariform shape. The sequence shown in fig. 15 must therefore frequently occur as a result of these two lines of specialization occurring at the same time with slightly different rates. The transition is accordingly of no particular evolutionary consequence. In the more primitive vessel segments, before sufficient time has elapsed for the primitive scalariform pits to break down into the opposite condition, the transition is truly palingenetic (fig. 13). The course of events is quite naturally the same, if one considers transitions from the lateral to the end wall. The interpretation of BOODLE and

WORSDELL is therefore incorrect, and affords an illustration of the dangers encountered when it is considered a sound procedure to interpret a sequence without first determining whether it is truly palingenetic and which end of the sequence is primitive.

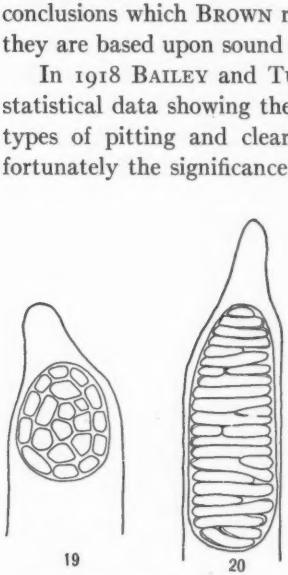
In 1917 JEFFREY (**13**) accepted the theory advanced by BOODLE and WORSDELL. He based his conclusions entirely upon positional sequences of the type shown in fig. 15, and argued that opposite pitting is more primitive than scalariform because the sequence shows that opposite pits fuse to form scalariform pits. He figured and described the truly palingenetic sequence (fig. 13) in *Liriodendron*, and argued that this transition is not primitive since the former sequence proves that opposite pitting is primitive. If one used the same line of argument and started with the *Liriodendron* transition the conclusions would be exactly reversed, and by chance correct.

In 1918 THOMPSON (**20**), after an intensive study of the Gnetales, confirmed the opinion of BOODLE and WORSDELL regarding the evolution of the vessel in this group, but suggested that the angiospermous vessel might have developed from a scalariform type of tracheid by the loss of the pit membranes. Since THOMPSON was able to show, without deciding that this theory was correct, that the further specialization of the vessel segment in the angiosperms was quite different from that in the Gnetales, he allowed the matter to rest.

In the same year BROWN (**7**) published evidence to show that the primitive vessel segment, as is the case in *Pteris*, is merely a modified scalariform tracheid, similar to those found in *Tetracentron*. BROWN'S paper is little more than a preliminary report, without illustrations, and it is a little difficult to understand why he did not present his evidence in detail in a later paper. He found transitions, in the dicotyledons, from the scalariform tracheid to the scalariform vessel segment, which he interpreted as a reflection of the phylogenetic origin of vessels. He also argued that scalariform lateral pitting is primitive since it is characteristic of conservative regions, since it is more characteristic of primitive families, and since transitions from scalariform to multiseriate lateral pitting are common in the angiosperms from the pith outward. The writer feels that the first two of the last three arguments have no foundation in fact, and that

the last argument is worthless unless it can be shown, on independent grounds, that scalariform lateral pitting is primitive. While the conclusions which BROWN reaches are correct, one cannot feel that they are based upon sound evidence.

In 1918 BAILEY and TUPPER (3) presented carefully compiled statistical data showing the true relationships between the various types of pitting and clearing up many important points. Unfortunately the significance of their results was overlooked by succeeding investigators, otherwise the whole question would have been settled several years ago.



FIGS. 19, 20.—Fig. 19, *Brysonima lucida*, gnetum-like type of perforation; fig. 20, *Andromeda ferruginea*, perforation with branching bars.

Miss BLISS (5) upheld the theory advanced by BOODLE and WORSDELL and JEFFREY, and presented the same arguments. She also suggested a new type of origin for the vessel in which the vessel originated by the haphazard fusion of circular pits. This process is supposed to have given rise to vessel segments with the curious netlike perforations (fig. 19) similar to those of *Gnetum*. If this theory were correct this type of perforation would be characteristic of scalariform woods, which she admits are more primitive than scalariform-porous woods. As a matter of fact she draws her illustrations largely from scalariform-porous woods, and a study of a great number of scalariform and scalariform-porous woods shows that this type of perforation is found almost exclusively in the latter group. In the seventeen species previously cited, with very long vessel segments, the *gnetum*-like perforations do not occur.

Miss BLISS also considers that the scalariform perforation with branched bars is a form of the netlike perforation, caused by the haphazard fusion of certain circular pits and the regular fusion of other circular pits (fig. 20). The branching of bars is characteristic of all types of scalariform perforations, and is, as BROWN (7) sug-

gests, merely a result of the formation of scalariform perforations from irregular scalariform pitting.

In 1921 Miss MACDUFFIE (15) gave further illustrations in favor of the theory advanced by Miss BLISS. She found that the netlike perforations were characteristic of the Compositae, among other highly specialized groups, and on this basis one would be forced to conclude that the Compositae possessed a very primitive type of stem or grant that the netlike type of perforation was highly specialized.

In 1923 THOMPSON (21) again attacked this problem and confirmed the views of BROWN. Although he overlooked the significance of the study by BAILEY and TUPPER, THOMPSON based his conclusions upon a study of a number of species, and found, as the writer has found, that there is no evidence that the netlike type of perforation is primitive. It is evidently an anomalous oddity resulting from the breaking down of vestigial scalariform perforations in scalariform-porous woods.

That the matter was still unsettled in 1925 is evident from the account of the origin of the vessel given by EAMES and MACDANIELS (10), which can be interpreted in favor of either theory.

In the literature concerning *Trochodendron*, *Tetracentron*, and *Drimys* there is considerable diversity of opinion. GROOM (12) suggested that these genera were degenerate and had at one time possessed true vessels. THOMPSON and BAILEY (22) came to the conclusion that these genera represent a very primitive condition and have never possessed vessels. JEFFREY and COLE (14) questioned the conclusions of THOMPSON and BAILEY, and reached a position similar to that suggested by GROOM. In 1918 BAILEY and THOMPSON (4) reviewed the entire question and confirmed their earlier results, and later BAILEY and TUPPER (3) gave the average lengths of the tracheids of these forms.

JEFFREY and COLE found in an injured root of *Drimys* an element, without perforations, but with scalariform lateral pitting which they considered an indication of the previous possession of vessels in these genera. At the time they were not informed of the normal occurrence of scalariform tracheids in the secondary xylem of this group. It is difficult to understand why they should

reason that scalariform lateral pitting is typical of a vessel and not of a tracheid, since they must have been aware of the very wide occurrence of scalariform tracheids in both fossil and recent plants. It would seem rather, if one is to use the principle of reversion, that the only justifiable conclusion is that the ancestors of these extraordinary types possessed a secondary xylem composed of scalariform tracheids. Since their whole argument depends upon this doubtful interpretation and the erroneous conception that opposite pitting is more primitive than scalariform pitting, their results cannot be taken as justifying the conclusion that these forms once possessed vessels.

Highly specialized woods occur in the Cactaceae and Crassulaceae in which vessels are partially or almost completely suppressed, due to adaptation to extreme environmental situations. In cases of this nature the vessel segments are replaced by peculiar structures which are short and quite obviously specialized, and which retain evidences of their origin from vessel segments. There is no similarity between woods of this type and the secondary xylem of *Trochodendron*, *Tetracentron*, and *Drimys*. It would be a remarkable reduction which resulted in regular and very long scalariform tracheids from short vessel segments in large arborescent plants. BAILEY and THOMPSON give a complete discussion of the evidence presented by JEFFREY and COLE in favor of the interpretation that these genera are truly primitive.

Summary

1. Tracheids are characterized by great average length, small cross-sectional diameter, angularity of outline, evenly thickened walls, thin walls, and the absence of a distinct end wall.

2. Vessel segments which retain these primitive characteristics nearly always have scalariform perforations; the scalariform perforation is therefore primitive.

3. There is a high correlation between the diffuse arrangement of vessels and the scalariform condition of the end wall.

4. Vessel segments with scalariform perforations are characterized by scalariform lateral pitting; therefore scalariform lateral pitting is primitive, and this leads to the natural inference that the

tracheid type from which the vessel was derived was also scalariform.

5. A high correlation was found between the scalariform condition of the lateral walls of vessel segments and the presence of bordered pits in the fibrous elements.

6. There was some evidence to indicate that a sequence from the protoxylem to the secondary xylem would reflect the origin of the vessel.

7. Many primitive woods show, in this positional sequence, all transitions from scalariform tracheids to scalariform vessel segments.

8. The evidence would indicate that vessel segments with scalariform pitting on both the end and side walls are more primitive than vessel segments with scalariform pitting on the end walls and opposite to alternate pitting on the side walls.

9. The wood of the vessel-less angiosperms *Trochodendron*, *Tetracentron*, and *Drimys* is unquestionably very primitive. The primitive tracheids of these genera resemble, to a considerable degree, the scalariform tracheids characteristic of the primary wood of primitive angiosperms.

The writer expresses his appreciation to Dr. I. W. Bailey for many helpful suggestions, and is particularly grateful to him for the use of valuable unpublished data.

BUSSEY INSTITUTION
BOSTON, MASS.

[*Accepted for publication February 27, 1929*]

LITERATURE CITED

1. BAILEY, I. W., The cambium and its derivative tissues. Amer. Jour. Bot. 7:355–367. 1920.
2. ———, Some salient lines of specialization in tracheary pitting. Ann. Botany 39:587–598. 1925.
3. BAILEY, I. W., and TUPPER, W. W., Size variation in tracheary cells. Proc. Amer. Acad. 54:150–204. 1918.
4. BAILEY, I. W., and THOMPSON, W. P., Additional notes upon the angiosperms *Tetracentron*, *Trochodendron*, and *Drimys*. Ann. Botany 32:503–512. 1918.
5. BLISS, MARY C., The vessel in seed plants. BOT. GAZ. 71:314–326. 1921.

6. BOODLE, L. A., and WORSDELL, W. C., Some points in the anatomy of Casuarinaceae and Gnetaceae. Ann. Botany **8**:231–264. 1894.
7. BROWN, F. B. H., Scalariform pitting a primitive feature in angiospermous secondary wood. Science N. S. **48**:16–18. 1918.
8. ———, Origin of the Hawaiian flora. Bishop Mus., Spec. Publ. **7**:138. 1921.
9. DE BARY, A., Comparative anatomy of the phanerogams and ferns. Oxford. 1884.
10. EAMES, A. J., and MACDANIELS, L. H., An introduction to plant anatomy. New York. 1925.
11. GEGENBAUR, C., Morphologisches Jahrbuch. **14**: p. 5. (transl. by W. B. SCOTT, The theory of evolution). New York. 1918.
12. GROOM, P., Remarks on the ecology of the Coniferae. Ann. Botany **24**: 241–269. 1910.
13. JEFFREY, E. C., The anatomy of woody plants. Chicago. 1917.
14. JEFFREY, E. C., and COLE, R. D., Experimental investigations on the genus *Drimys*. Ann. Botany **30**:359–368. 1916.
15. MACDUFFIE, R. C., Vessels of the gnetalean type in angiosperms. BOT. GAZ. **71**:438–445. 1921.
16. VON MOHL, H., Grundzüge der Anatomie und Physiologie der vegetabilischen Zelle. Braunschweig. 1851.
17. MORGAN, T. H., A critique of the theory of evolution. Princeton. 1916.
18. SCOTT, W. B., The theory of evolution. New York. (p. 61). 1918.
19. THOMPSON, D. W., Growth and form. Cambridge. 1917.
20. THOMPSON, W. P., Independent evolution of vessels in Gnetales and angiosperms. BOT. GAZ. **65**:83–90. 1918.
21. ———, The relationships of the different types of angiospermic vessels. Ann. Botany **37**:183–192. 1923.
22. THOMPSON, W. P., and BAILEY, I. W., Are *Tetracentron*, *Trochodendron*, and *Drimys* specialized or primitive types. Mem. N.Y. Bot. Gard. **6**:27–32. 1916.

COMMON FOSSIL POLLEN OF THE ERIE BASIN[1]

PAUL B. SEARS

(WITH PLATES I–III)

Introduction

This paper is intended chiefly to assist in the identification of common and significant pollen found embalmed in bog deposits in north-central United States. Before proceeding with the actual key and description, a brief explanation seems to be in order.

For a number of years European botanists have been counting the various species of pollen found at different levels in bogs. Such statistics have been used to trace the course of plant succession and the climatic changes of which it is held to be the expression. STARK (9) has prepared a concise summary on the subject up to about 1924; ERDTMAN (3) has arranged the literature preceding 1927; while FULLER (5) has twice noted for American students the general results in this field. Work continues actively in Europe and is under way in the United States.

ERDTMAN'S remark that the work is not easily done is quite true. He states: "It includes field work often wet and rough, a most minutieuse laboratory work, designing of diagrams, and study of [an extensive and widely scattered] literature." The value of such work rests upon two facts: (1) the form of pollen is, with certain reservations, definite for a species; (2) the precise structural details of pollen coats are often well preserved, not only in peat but in the silts associated with it.

Difficulties in applying these two facts arise, however, from several sources: (1) there are no comprehensive manuals of pollen structure; (2) pollen grains in bogs act as centers for flocculation of organic and inorganic materials and are not always easily loosened from this matrix; (3) it is not always possible even for an expert to distinguish related genera by their pollen, much less species of the same genus; (4) there are a number of cases of accidental resem-

[1] Contribution from the Botanical Laboratory, University of Oklahoma, N. S., no. 2.

blance between pollen of widely separated families; (5) not all pollen is equally well preserved, for example, it is well known that the pollen of *Juniperus* quickly breaks down; (6) some species of trees might contribute pollen quite out of proportion to nearness and abundance of the species. The phenomenon known as "sulphur showers" consists of heavy wind deposits of pine pollen, often at considerable distance from pine forests.

In spite of these difficulties, the practical results already achieved seem to justify the effort being devoted to pollen statistical analysis.

Technique

Methods employed by European workers are discussed briefly in the paper by STARK (9), and more at length in the one by FURRER (6). The latter paper includes much critical discussion of methods and possible sources of error.

For the work done in this laboratory, samples have been secured, both with an ordinary soil auger extended by means of threaded pipe, and by means of a Davis peat sampler obtained from Eberbach & Son of Ann Arbor. This latter instrument has the advantage of supplying a continuous series of 6-inch cores to a depth of over 20 feet. The Swedish peat borer used in Europe is calibrated on a metric basis, which in some respects is an advantage. No matter what type of sampler is used it is necessary carefully to avoid contamination. In sampling a given bog attention should be paid to its profile, and samples should be taken in duplicate columns from representative portions. Samples are best preserved in glass tubes of appropriate size with corks at either end. If possible it is an advantage to mount the material soon after collecting; if not, some method of preventing bacterial action and molding should be used.

Laboratory preparation varies considerably with the character of the specimen. The principal object is to loosen the pollen grains from the colloidal substance which tends to gather about them and form an opaque mass. The usual method of accomplishing this is to place a piece of material not larger than 10 mm. in diameter in a 10 per cent solution of KOH and boil it down. An excess of glycerin is added, after which the material can be mounted, ringed, and studied. The KOH treatment has the advantage of imparting a distinct yel-

lowish to reddish stain to the grains, but it can also cause disintegration of grains unless watched carefully. Wherever the condition of the peat permits, a preferable method is deflocculation by means of ammonia water, immersing the tubes in a water bath. In such cases, by the time one is ready to add glycerin the greater portion of the alkali has volatilized. In this laboratory we have generally used glycerin-lactic acid-phenol rather than pure glycerin as a mounting medium (2), while glycerin jelly is valuable for permanent mounts. It is also often useful to presoak the specimens, either in clean distilled water or in ammonia water. Some European workers have used what seems like rather severe treatment, that is, strong acids, centrifuging, washing, etc., but with good results, notably in the case of sandy peats and interglacial material. On the whole, however, it is a safe rule to use the mildest treatment which will completely loosen all pollen from the floccules.

In mounting it is well to remove all sand particles, etc., before covering and ringing. This will permit no. 1 covers to be used, which are desirable, since oil immersion, or better, water immersion may be essential in critical identifications.

In counting, it is sound practice not to base estimates on counts of less than 100 grains. Sampling and mounting from the same sample should be done in duplicate series where possible, and care should be taken to prevent contamination by fresh pollen.[2] If the drops mounted are small and the pollen abundant, the euscope or similar projecting device greatly relieves the fatigue of counting. In addition to counts and percentage calculations, the pollen frequency (PF) or total number of pollen grains per square cm. of slide may be recorded; but there is no way, because of the variations of peat density, necessary treatment, etc., actually to standardize results at present.

MEINKE (8) and ERDTMAN (4), among others, distinctly emphasize the need for a comprehensive knowledge of pollen in general on the part of those attempting pollen statistical work. It is essential that the worker read WODEHOUSE's discussion of the problems of

[2] To realize the force of this statement, simply expose a slide rubbed with olive oil on the table during a summer day. Examination will generally reveal a considerable number of grains.

pollen structure and classification (10). The illustrated papers of ERDTMAN (4), DOKTUROWSKI (1), and MEINKE (8) deal with European species, but within certain limits they contain much useful information on American genera, occasionally on American species.

The report which follows is based upon studies of modern pollen, stained with aqueous methyl blue according to the method described by WODEHOUSE (10). The forms described were selected principally with reference to those actually found by the writer in peats; others are included on the basis of field studies in living bogs, and some on account of their abundance in the air. The literature dealing with bogs of eastern North America has also been canvassed and certain anemophilous species noted for inclusion here. The following key deals mostly with genera, and occasionally with families. Safe distinctions beyond that point, if possible at all, involve minutiae which would make the key either misleading or unwieldy. It should not be forgotten that pollen from one anther may display a considerable range of variation in size, and even in marking and form. Injuries, accidental compression, position on slide, and (in folding pollen) the degree of expansion, all have a marked effect on appearance. It is not surprising that even with the greatest care and the help of specialists there should be pollen found whose identity is uncertain. To have a certain number of unknowns in a pollen census may therefore be regarded as a mark of care rather than the reverse. To offset this situation it may be added that most of the copious producers of anemophilous pollen in the bog series have rather distinctive generic characters, difficulties being encountered mainly among the Betulaceae, Gramineae, and Cyperaceae.

Key to pollen

1. Pollen with two air sacs 2
   Pollen without air sacs 4
2. Total length at least 100 μ ..... *Abies* (I, 1)[3]
   Total length less than 90 μ 3
3. Sac short-conical in lateral view, total length about 75–85 μ *Picea* (I, 2)
   Sac globular or ellipsoid, total length about 75 μ or less *Pinus* (I, 3, 3a)

[3] Roman numerals in parentheses refer to paragraphs in descriptive notes following; arabic numerals to figures in plates.

4. Pollen grains in tetrads 5
   Pollen grains separate 6
5. Tetrad[4] very compact, common wall thick, finely pitted above each grain ..... *Juncus* (X, 16)
   Tetrad loose, no common wall, exine of each grain distinctly pitted and with 1 large pore (monopored) ..... *Typha* (IV, 7)
6. Grains without distinct perforate pores or slits (expansion folds) ... 7
   Grains with noticeable pores or slits (expansion folds) 12
7. Pollen subglobular to prismatic or pyramidal, exine with 1 or more inconspicuous roughened exits (germ pores)

   Cyperaceae { *Carex* (IX, 12) / *Eriophorum* (IX, 13) / *Eleocharis* (IX, 14) / *Scirpus* (IX, 15) }

   Pollen spherical or subglobular, exine with no trace of roughened exits 8
8. Exine distinctly reticulate or papillate 9
   Exine smooth (psilate) or finely punctate only 10
9. Exine reticulate, diameter 21–30 μ ..... *Potamogeton* (VI, 9)
   Exine coarsely pebbled (subechinate) 60–75 μ ..... *Tsuga*[5] (II, 6, 6a)
10. Exine finely punctate, interrupted or broken .... *Populus* (XIII, 21)
    Exine smooth (psilate) occasionally torn 11
11. Diameter 15–20 μ ..... *Juniperus*[6] (II, 5)
    Diameter about 60 μ ..... *Larix* (II, 4)
12. Pores one, with or without an operculum 13
    Pores or expansion folds more than one 15
13. Surface of exine smooth ..... Gramineae (*Glyceria*) (VIII, 11)
    Surface of exine reticulate, pitted or pebbled 14
14. Surface reticulate, diameter about 20 μ ..... *Typha* (IV, 7)
    Surface pebbled (subechinate) ..... *Sparganium* (V, 8)
15. Pores grouped at one side of grain 16
    Pores more or less uniformly spaced 17
16. Pores 10 or more aspidate or mammillate ... *Juglans* (XVII, 32, 32a)
    Pores 3 simple, distinct, diameter about 50 μ .... *Carya* (XVII, 33)
17. Pores variable but numerous, usually 10 or more 18
    Pores fewer, usually 5 or less 21

[4] *Drosera* and Ericaceae show tetrads, but of distinctly different type (MEINKE 8).

[5] *Osmunda* and other pteridophytes have spores somewhat similar in type, but with triradiate lines.

[6] But *Juniperus* is seldom or never preserved for long.

18. Pores large, pollen diameter seldom less than 25 μ 19
Pores small, pollen diameter generally less than 25 μ
Amaranthus (XIX, 35)
Chenopodium (XIX, 36)

19. Surface spiny (echinate) pores irregular ..... *Sagittaria* (VII, 10)
Surface smooth (psilate) or nearly so 20

20. Pores about 10, their margin irregular ..... *Plantago* (XXI, 38)
Pores about 20, with clean edges, pore lids visible in fresh material ..... *Liquidambar* (XII, 18)

21. Pores 3 to 5 not in expansion folds and surface never spiny (echinate) 22
Expansion folds present 25

22. Pores "aspidate" or apparently so, that is, with shieldlike or mammillate thickening about them 23
Pores not aspidate 24

23. Pollen large (35×25 μ), compressed, punctate, pores resembling depressions in locally thickened exine ..... *Tilia* (XI, 17, 17a)
Pollen smaller (20–30 μ) not compressed, pores perforate
Betulaceae { *Betula* (XIV, 22); *Alnus* (XIV, 23, 23a, 23b)
Corylaceae { *Corylus* (XIV, 24, 24a); *Ostrya* (XIV, 25, 25a); *Carpinus* (XIV, 26, 26a)

24. Pores 5, grain slightly pentagonal, faintly reticulate with broad shallow ridges ..... *Ulmus* (XV, 29, 29a)
Pores 3, not projecting but sometimes with border in fresh material due to thickening of intine below ..... *Celtis* (XV, 30)

25. Exine faintly reticulate or echinate 26
Surface smooth (psilate) or at most finely pitted or papillate, never reticulate or echinate 31

26. Surface thickly set with sharp spines ..... *Ambrosia* (XXII, 39)
(spines in *Iva* not so sturdy as in *Ambrosia*) ..... *Iva*
Surface reticulate 27

27. Pores distinct within prolonged expansion folds appearing midway between apices of triangulate grain in polar view
Rumex (XX, 37)
Pores not distinct within ragged expansion folds or if so, grain over 40 μ in diameter 28

28. Folds, usually 4, not prolonged toward poles of grain which is often over 20 μ diameter ..... *Fraxinus* (XVIII, 34, 34a)
Folds, usually 3, prolonged toward poles 29

29. Reticulations increasing in size toward equator, periphery of grain appearing roughened.....*Salix* (XIII, 20, 20a)
Reticulations minute, somewhat uniform, periphery of grain fairly entire.....*Platanus* (XII, 19)[7]
30. Exine slightly thickened at edges of folds, grain compressed, about 50×40 μ.....*Fagus* (XIV, 27, 27a)
Exine not perceptibly thickened, diameter less than 40 μ.....31
31. Exine becoming very thin at margins of folds, smooth to finely rugose, grain not compressed.....*Acer* (XVI, 31, 31a)
Exine neither thicker nor much thinner at folds, surface punctate, grains oblate spheroid.....*Quercus* (XIV, 28, 28a, 28b)

Descriptive notes

I. CONIFERS.—The pollen of *Abies*, *Picea*, and *Pinus* all have two lateral air sacs or wings, and differ principally in size, that of *Abies* (*balsamea*) being about 125 μ long and 75 μ high; *Picea* (*mariana*) 85 μ long and 55 μ high; while the pines vary 60–75 μ in length and 30–35 μ in height. *P. banksiana* in these samples is about 60×30 μ, *P. strobus* about 60×30 μ, and *P. resinosa* about 75×35 μ. There are in addition slight differences in pattern and relative shape of parts, but because of the difference in position and frequent distortion of fossil material when mounted, size seems to be the safest criterion (FURRER 6). The markings (reticulations on wings, mazes and pits on central portion) are inside the coat.

II. LARIX, JUNIPERUS, THUJA.—These are similar in type, being globular without pores or distinct marking. But *Larix* is about 65 μ in diameter while the two others are considerably smaller. Of the three, *Larix* is the only one certainly found in peat, where it often retains one or more globules of its typical resinous contents. *Juniperus* and *Thuja* break down quickly when wet. *Tsuga* is an important pollen, likely to be confused with pteridophyte spores except that it lacks the triradiate prismatic faces on the inside of the latter. It is turtle-shaped, about 70 μ long with a convex warty or subechinate outer surface and a smaller, thin-walled, often sunken inner surface. Seen from above or below it of course appears globular.

III. MONOCOTYLEDONS.—Generic distinctions are often impossible, but as a rule monocotyledonous pollen of the more abundant

[7] May be confused with *Quercus*, *Salix*, *Fraxinus*.

kinds can be placed in the proper family, and at any rate is distinct from most dicotyledonous pollen.

IV. TYPHACEAE.—The statement is made that pollen of *Typha latifolia* is in close tetrads, that of *T. angustifolia* in linear; but the writer has found it as single grains in both. The surface is finely reticulate as in *Potamogeton*, but differs in possessing a single apical pore of moderate size. The single grain is about 20 μ in diameter.

V. SPARGANIACEAE.—*Sparganium* (*eurycarpum?*) is a globular pollen of about 25 μ, combining the monopored character of Graminaceae and a granular thin surface like the exits in Cyperaceae.

VI. ZANNICHELLIACEAE.—*Potamogeton:* this group requires further study, but *P. richardsonii* shows a small elongate thick-walled grain with distinct reticulations and no discoverable pore. Dimensions 20–25 μ. Except for smaller size of areolae and absence of pore it suggests a grain of *Typha* type.

VII. ALISMACEAE.—*Sagittaria* sp.: globular subechinate, 10-pored, with short spiny pattern continuous over the pores. In one way this suggests the pebbly and warty exits of *Carex*, but it is perhaps more specialized, with a central spine in each pore not unlike the operculum of grasses. The several poral spines about this suggest a ragged fringe.

VIII. GRAMINEAE.—As pointed out by WODEHOUSE (10), generic distinctions are difficult in this family, but the family character is unmistakable. Pollen globular to ovate, varying greatly in size but all monopored psilate (1-pored, smooth). The distinct central operculum may be absent in fossil material.

IX. CYPERACEAE.—The form of pollen in this family may vary from globular to pyramidal. The exine character however is unmistakable. At one end is a thin-walled warty or pebbly area, and in all forms examined (*Carex, Scirpus, Eleocharis, Eriophorum*) there are from one to three similar areas elsewhere on the exine, harder to distinguish than that of the end. In consulting the key and drawings it should be remembered that further work needs to be done on this difficult group before determinations can safely be made. The outline drawings of MEINKE (8) should be consulted if possible.

X. JUNCACEAE.—This group has not been studied adequately, but *Juncus effusus* shows a tight tetrad with a thick more or less hyaline

wall and a faintly broken area as in *Carex*, above each of the four protoplasts.

XI. DICOTYLEDONS.—Tiliales: the pollen of *Tilia* (*americana* or *europea*) is very typical. It is flattened, triangulate, with three median pores, each appearing like a pit in the locally thickened exine. Surface of exine finely pitted. Diameters about $35 \times 25\ \mu$.

XII. HAMAMELIDALES.—The two genera *Liquidambar* and *Platanus* are in different families and possess distinctly different types of pollen. That of *Liquidambar* has about ten pores with lids, no expansion folds, is about $35\ \mu$ in diameter, with nearly smooth surface. In fossil material with lids gone it might be confused with chenopodiaceous pollen except for its larger size. Pollen of *Platanus* is tricolpate, finely reticulate, and about $20\ \mu$ in diameter. It may be confused with pollen of *Quercus* or *Salix*. It appears to show flecks of exine over the furrowed surface, which may be homologous to the flecked lids in *Liquidambar*.

XIII. SALICALES.—*Populus* has a very simple globoid pollen with a thin granular interrupted exine, and no pores. It is distinguished from *Juniperus* by its larger diameter (about $30\ \mu$) and exine character. *Salix* has a tricolpate grain ($20\ \mu$ in diameter) with small reticulations at the poles and larger ones at the equators. Folds often appear median in polar view (cf. especially *Fraxinus* and *Platanus*).

XIV. FAGALES.—The pollen of American forms of this order require critical study because of their extreme importance in pollen analysis. For European forms see JENTYS-SZAFER (7). The Betulaceae (*Betula*, *Alnus*) and Corylaceae (*Corylus*, *Ostrya*, *Carpinus*) are readily distinguished from the Fagaceae (*Fagus*, *Quercus*) by the mammillate or blister-like thickened exine at each pore ("aspidate"[8]) in the first two families, and the presence of three expansion folds in the last. Beyond these limits, however, determination of genera is difficult. The present key makes no attempt to separate the Betulaceae from the Corylaceae, or to distinguish their genera. The pollen of *Fagus* ($50 \times 40\ \mu$) is larger than that of *Quercus* ($30 \times 20\ \mu$), but both are, unlike *Acer*, compressed at the poles. *Fagus* often shows distinct thickening of the exine along the expansion fold, while that of *Quercus* does not. It should be noted that the

[8] WODEHOUSE, unpublished.

aspidate character is marked in *Juglans* and perhaps suggested in *Celtis*, but other characters make the distinction of these genera from *Betula*, etc., possible.

XV. URTICALES.—*Ulmus* is a distinctly pentagonal grain of about 30 μ diameter in polar view, but appears ovoid in lateral view, being flattened. There are five oval pores, one at each angle of the equatorial line, and the surface is distinctly undulate because of large shallow areolae. Exine is thickened about the pores. *Celtis* likewise has equatorial pores, but only three and the grain is quite globular. It is about 30 μ in diameter and may appear aspidate when fresh, due to a lenslike thickening of intine below the pore.

XVI. SAPINDALES.—*Acer saccharum* (32–38 μ) would be confused with the tricolpate (three-furrowed) oak, except for its globular form, finely striated surface, and the fact that its exine becomes extremely thin at the edges of the folds. This last character seems to hold in all species of *Acer* examined. It must be remembered that there is a great mass of (at present) nondescript tricolpate pollens of herbaceous and shrubby plants, but fortunately few are abundantly wind-borne.

XVII. JUGLANDALES.—In *Carya* and *Juglans* the distribution of pores (three in *Carya*, ten or more in *Juglans*) is asymmetrical. The pores of *Juglans* are distinctly aspidate, making this pollen (of 35 × 30 μ) easy to determine. Those of *Carya* appear as plain perforations, lying on a smaller circle than the equator of the very large grain (50–55 μ).

XVIII. LOGANIALES.—The pollen of *Fraxinus* is very similar to that of *Salix* and *Platanus*, but is usually quadrangulate oblate, and the four (or three) apical expansion furrows of the reticulate grain are short. Dimensions about 25 × 20 μ.

XIX. CHENOPODIALES.—The pollen of this order is well known and distinctive, being globular, psilate (smooth), without expansion folds but with numerous perforate pores each having a basal membrane faintly flecked. The pore number varies so much within genera that no attempt can be made to separate Chenopodiaceae and Amarantaceae.

XX. POLYGONALES.—*Rumex* is of the familiar tricolpate type, but there is a distinct equatorial pore in each fold. The folds lie between, not at, the blunt angles of the equator and are long. Exine reticulate; diameter about 25 μ.

XXI. PLANTAGINALES.—*Plantago* is abundant in modern air and resembles *Sagittaria* (q.v.) in size (25–30 μ), number (ten), and ragged margins of pores, but is smooth instead of spiny.

XXII. ASTERALES.—Composite pollen is thick-walled, tricolpate, but differs greatly in details of pattern. *Ambrosia* and perhaps *Iva* are the two most likely to be of interest in anemophilous deposits. General characters are clear from key and figure, but further information should be secured, if desired, from the papers of WODEHOUSE, especially (10).

The writer wishes to acknowledge the kind assistance of numerous friends in the preparation of the present paper. Particularly is he indebted to Messrs E. H. WILSON, H. B. SEARS, G. W. BLAYDES, A. J. SHARP; Drs. R. P. WODEHOUSE, R. M. BALYEAT; and Miss HELEN VINCENT.

UNIVERSITY OF OKLAHOMA
NORMAN, OKLA.

[*Accepted for publication February 19, 1929*]

LITERATURE CITED

1. DOKTUROWSKI, W., Pollen im Torf. Mitt. wissensch.-experiment. Torfinstitut Moskau. (Russ.) no. 5. 33–44. 1923.
2. DRAPER, P. I., A demonstration of the technique of pollen analysis. Proc. Okla. Acad. Sci. **8**:63–64. 1928.
3. ERDTMAN, G., Literature on pollen statistics published before 1927. Geol. Foren. I. Stockholm Forhandl. **49**:196–211. 1927.
4. ———, Beitrag zur Kenntniss der Mikrofossilien. Ark. Bot. **18**:1–9. 1923.
5. FULLER, G. D., Peat bogs and postglacial vegetation. BOT. GAZ. **87**:560–562. 1929 (also *ibid.* **83**:323–325. 1927).
6. FURRER, E., Pollenanalytische Studien in der Schweiz. Beibl. Vierteljahrschr. Naturf. Gesell. in Zürich. **72**:1–38. 1927.
7. JENTYS-SZAFER, J., La structure des membranes du pollen de *Corylus*, de *Myrico*. Bull. Acad. Pol. Sci. Lett. Cl. Sc. Math.-Nat. B. Bot. 75–125. 1928.
8. MEINKE, H., Atlas und Bestimmungsschlüssel zur Pollenanalytik. Bot. Arch. **19**:380–449. 1927.
9. STARK, P., Der gegenwärtige Stand der pollenanalytischen Forschung (Sammelreferat). Zeitschr. Bot. **17**:89–125. 1925.
10. WODEHOUSE, R. P., The phylogenetic value of pollen grain characters. Ann. Botany **42**:891–934. 1928.

EXPLANATION OF PLATES I–III

All figures were drawn with camera lucida and Leitz microscope; details drawn under oil immersion where necessary. As reproduced all represent a magnification of 700 diameters, except fig. 27, which is about 450.

FIG. 1.—Abies balsamea, lateral view.

FIG. 2.—Picea mariana, lateral view, showing subconical air sacs.

FIG. 3.—Pinus strobus, lateral view; fig. 3a, top view.

FIG. 4.—Larix decidua, showing globule of resinous contents.

FIG. 5.—Juniperus virginiana; Thuja very similar.

FIG. 6.—Tsuga canadensis, lateral view (ventral side up) showing coarse projecting dorsal coat and thin ventral one; fig. 6a, ventral view.

FIG. 7.—Typha latifolia, typical close tetrad, but grains often occur singly.

FIG. 8.—Sparganium sp. showing single roughly operculate pore and pebbly surface.

FIG. 9.—Potamogeton richardsonii.

FIG. 10.—Sagittaria sp.

FIG. 11.—Glyceria borealis, single operculate pore and smooth surface.

FIG. 12.—Carex vulpinoides, showing, with the next three, typical granular exits of Cyperaceae.

FIG. 13.—Eriophorum virginicum.

FIG. 14.—Eleocharis palustris.

FIG. 15.—Scirpus americanus.

FIG. 16.—Juncus effusus.

FIG. 17.—Tilia americana, polar view; fig. 17a, equatorial view.

FIG. 18.—Liquidambar styraciflua.

FIG. 19.—Platanus occidentalis.

FIG. 20, 20a.—Salix sericea.

FIG. 21.—Populus deltoides.

FIG. 22.—Betula lutea.

FIG. 23, 23a, 23b.—Alnus incana.

FIG. 24, 24a.—Corylus americana.

FIG. 25, 25a.—Ostrya virginiana.

FIG. 26, 26a.—Carpinus caroliniana.

FIG. 27.—Fagus grandifolia, polar view (×450); fig. 27a, equatorial view (×700).

FIG. 28.—Quercus alba; fig. 28a, Q. macrocarpa; fig. 28b, Q. rubra.

FIG. 29, 29a.—Ulmus americana.

FIG. 30.—Celtis occidentalis.

FIG. 31, 31a.—Acer saccharum.

FIG. 32, 32a.—Juglans nigra.

FIG. 33.—Carya ovata, polar view.

FIG. 34, 34a.—Fraxinus lanceolata.

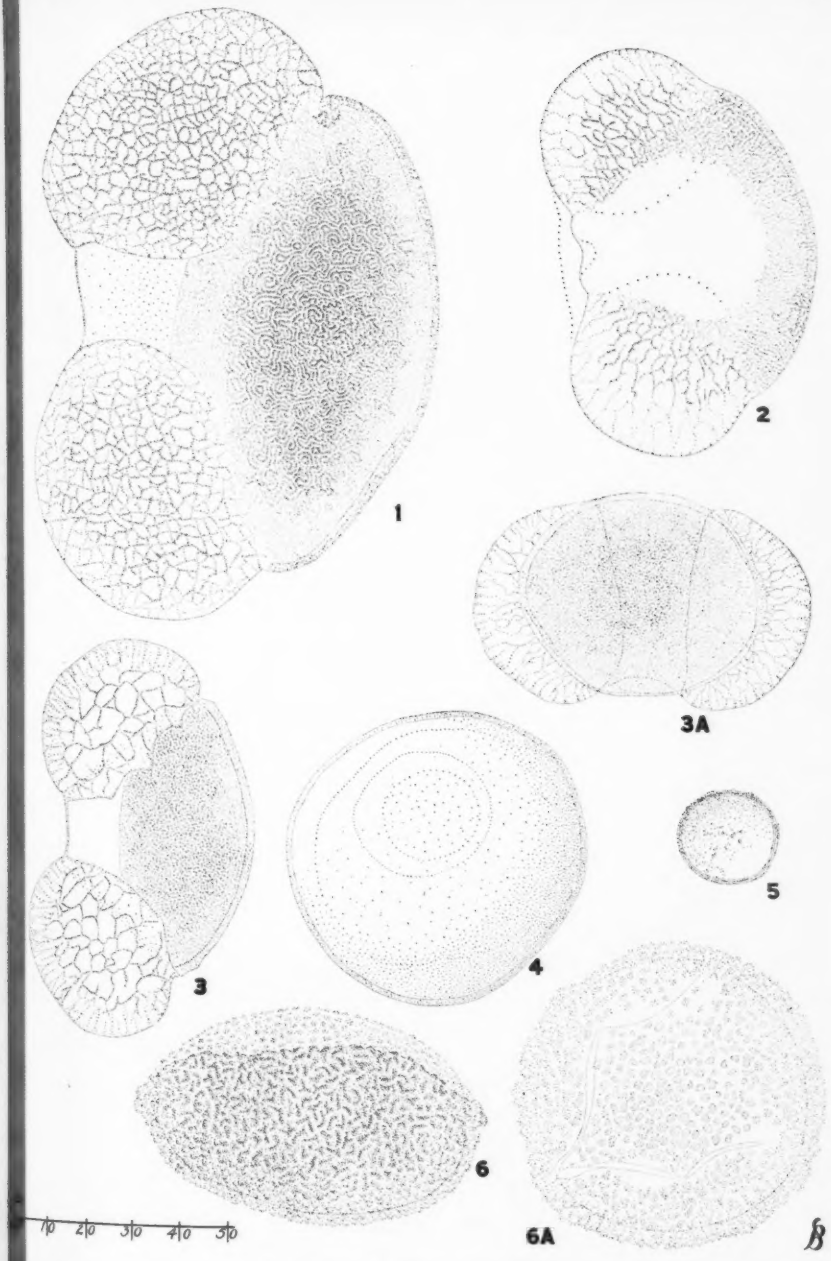
FIG. 35.—Amaranthus retroflexus.

FIG. 36.—Chenopodium sp.

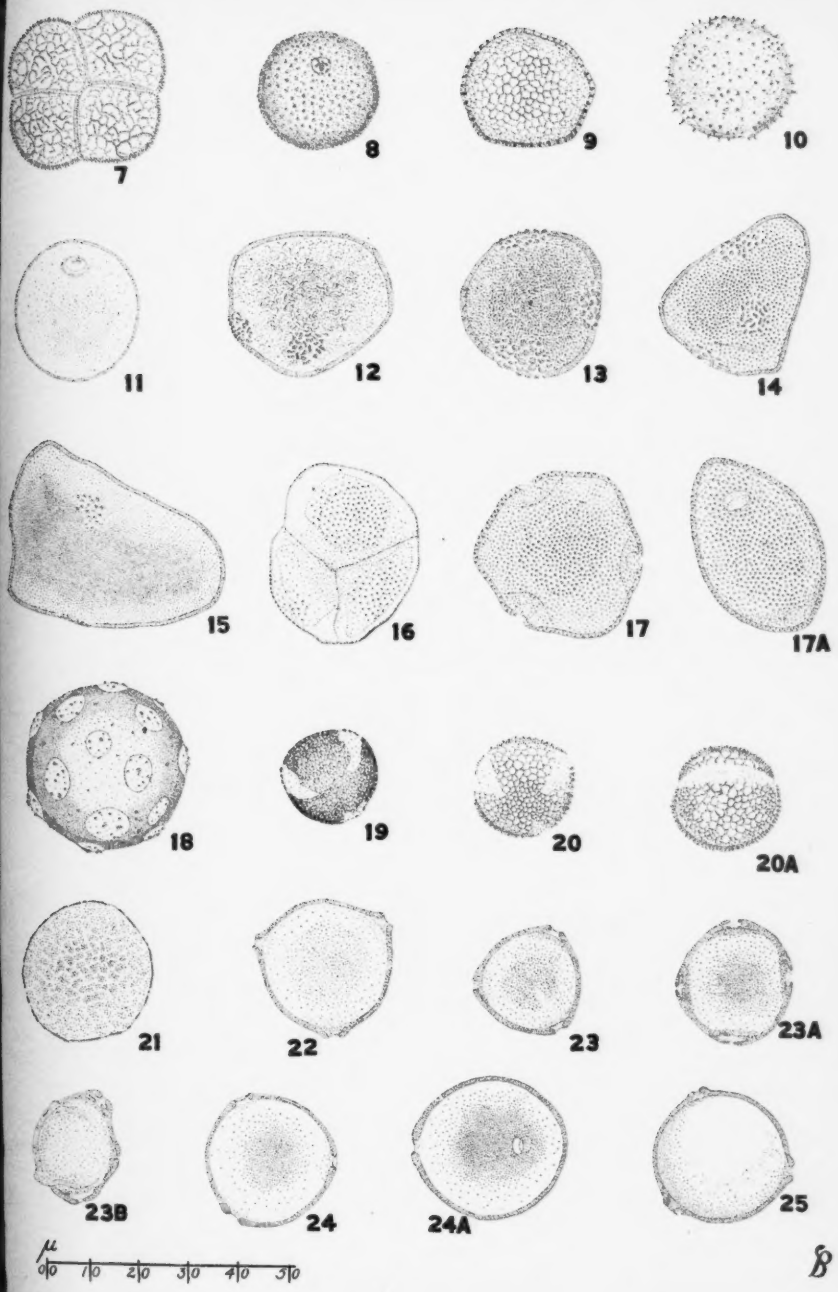
FIG. 37.—Rumex brittanica.

FIG. 38.—Plantago rugelii.

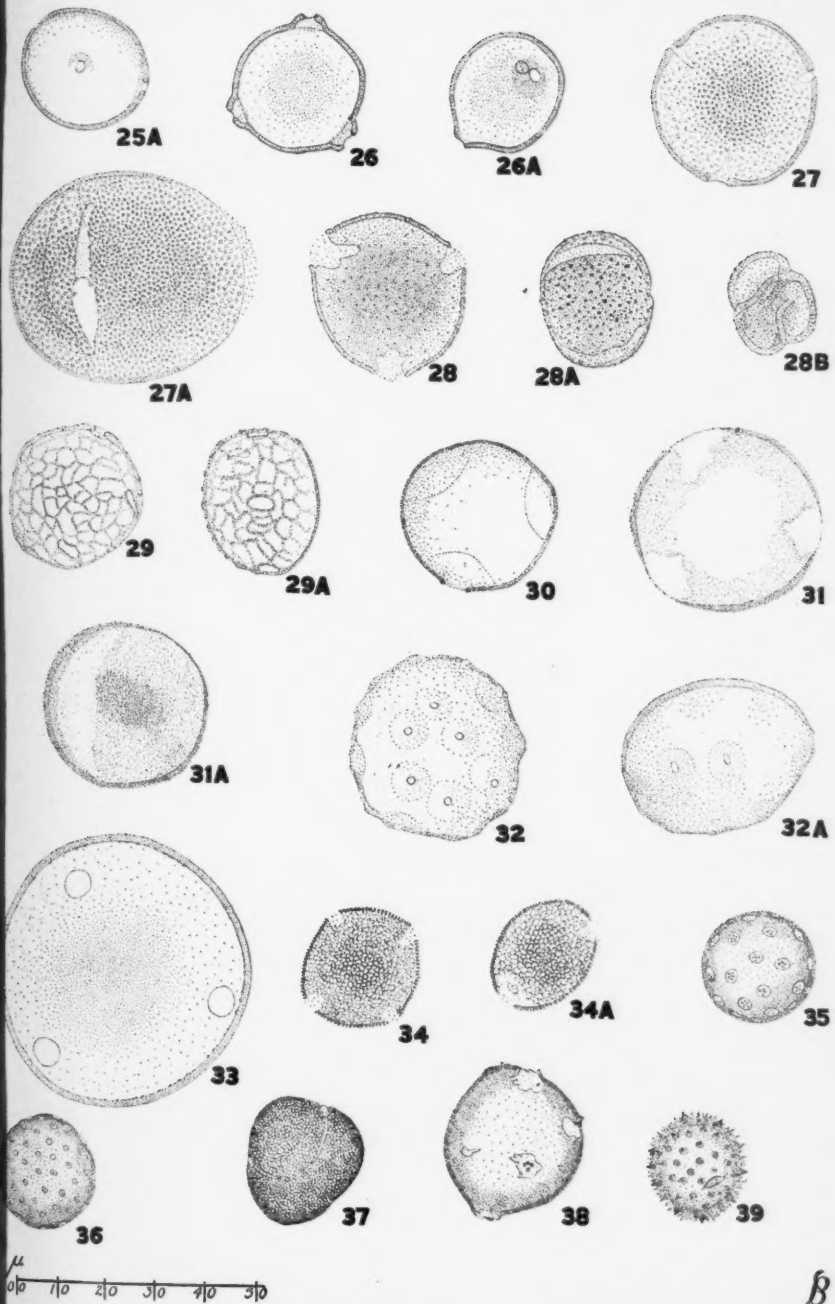
FIG. 39.—Ambrosia psilostachya.



SEARS on FOSSIL POLLEN



SEARS on FOSSIL POLLEN



SEARS on FOSSIL POLLEN

CURRENT LITERATURE

BOOK REVIEWS

New books on paleobotany

The two manuals here reviewed serve entirely different purposes. The French book[1] is a classroom syllabus. The German text,[2] of which only the first volume has appeared, is a large reference book which intends to summarize our present knowledge of fossil plant structure, in two profusely illustrated volumes. The first book is a great help in teaching paleobotany and the other one is indispensable for research. There is no up-to-date English textbook for a general introduction to paleobotany, and the literature on this subject is evenly divided between the three languages English, German, and French. No one can make a thorough study of paleobotany without a fluent command of these three languages. Of late a large number of botanical textbooks have been published in German, including some on paleobotany.[3]

A handy outline of paleobotany for American students was published by BERRY[4] in 1920, but is out of print now because it appeared in a government publication. The standard English textbook for students of fossil plant structures is, and will be for a long time, SCOTT'S work,[5] while the great reference book by Seward[6] attempts to give a complete account of fossil pteridophytes and gymnosperms, devoting only a small space to thallophytes and bryophytes. Some portions of SEWARD'S work are out of date, and the four volumes do not contain enough illustrations to fullfil the object of being a complete reference book.

HIRMER'S handbook compares favorably with the volumes by SCOTT and SEWARD, on account of the very large number of its well executed illustrations, and also because of its very complete bibliography. HIRMER attempts many

---

[1] BERTRAND, P., Conférences de Paléobotanique. 8vo. pp. 138. Librairie de l'enseignement technique, 3 Rue Thenard, Paris. Léon Eyrolles, Éditeur. 1926.

[2] HIRMER, M., Handbuch der Paleobotanik (with chapters by JULIUS PIA and WILHELM TROLL). Vol. I. Thallophyta, Bryophyta, Pteridophyta. 8vo. pp. xvi+708. *figs. 817*. Munich and Berlin: R. Oldenbourg. 1927.

[3] POTONIÉ, H., Lehrbuch der Paläobotanik. 2d ed. Berlin. 1921. GOTHAN, W., Paläobotanik. Berlin and Leipzig: Sammlung Göschen. 1920.

[4] BERRY, E. W., Paleobotany: a sketch of the origin and evolution of floras. Smithsonian Report for 1918. pp. 289–407. Washington. 1920.

[5] SCOTT, D. H., Studies in fossil botany. 2 vols. 3d ed. London. 1920–23.

[6] SEWARD, A. C., Fossil plants. 4 vols. Cambridge. 1898–1919.

reconstructions, and devotes considerable attention to the classification and organization of his material. His book is up-to-date and should now be used as a basis for any research undertaken in fossil plant structures. It is to be hoped that the second volume, which will contain the gymnosperms, will appear soon.—A. C. NOÉ.

Organography of plants

The appearance of a new edition of GOEBEL'S principal book[7] in which his morphological life work is summarized, is an historical event in botanical literature.

This new edition is considerably enlarged. The number of illustrations has been increased from 459 in the second edition to 621 in the third edition of part I; and there are about 100 more pages. The second edition appeared in 1913.

A. P. DECANDOLLE designated as the subject of his "Organographie végétale" (Paris 1827) the "Description raisonnée des organes," and he also included the anatomy. The latter is excluded for practical reasons in GOEBEL'S organography. GOEBEL distinguishes two problems in his organography; the first one is to discover how the many different forms of plant organs originate and what the relations between them are. GOEBEL gave the name morphology to this problem. The second problem of organography is, according to GOEBEL'S the investigation of the connection between plant forms and living conditions.

Part I deals with general organography. It considers the formation of organs in the different levels of the plant kingdom, with the problem of symmetry in the plant, the transformation, reduction, fusion, and partition of plant organs. In the last chapter the author discusses the relation of the formation of organs to internal and external factors.

GOEBEL'S conception of organography forms a very interesting section of botanical science. It is a cross-section through several divisions of standardized botany, and connects a great many facts which are customarily distributed among our conventional divisions morphology, physiology, and ecology. Probably its main value lies in the fact that plant life is studied in this volume from a different angle from that found represented in conventional classification of plant sciences.—A. C. NOÉ.

Fungi of North America

A very noteworthy contribution has been made by SEYMOUR,[8] in his monumental compilation of the fungi which have been reported to occur on North American hosts. It is fitting that this volume grew out of the early manuscripts of FARLOW'S, which were used in his edition of 1888–1891; that it is the result

[7] GOEBEL, K., Organographie der Pflanzen. Part I. Allgemeine Organographie. 8vo. pp. ix+642. *figs. 621.* Jena: Gustav Fisher. 1928.

[8] SEYMOUR, A. B., Host index of the fungi of North America. pp. xiii+732. Cambridge: Harvard University Press. 1929.

of the labors of his associate through all the years; that it was made possible by the interest and material assistance of the late Mrs. FARLOW; and that it is published by the Harvard University Press.

The volume contains 80,000 names of hosts and fungi. Since all systems are arbitrary, it is to be hoped that the system used here will tend to stabilize host and parasite names in the United States. An attempt is made to apply to fungi the International Rules of Nomenclature adopted at Brussels, which may not meet with entire approval by all systematists.

The volume will prove an invaluable addition to the library of every mycologist and phytopathologist. It is well printed on high grade paper.—G. K. K. LINK.

Mycorhiza

Biology is benefited by Miss RAYNER'S publication as a separate volume[9] of her papers on mycorhiza which appeared in the New Phytologist. This volume is of special appeal to those interested in plant nutrition, infection, and adaptation. Although the volume is essentially a review, it is more than a mere compilation, in that the material is critically evaluated. The author states in conclusion: "On the whole it may be said that the views so strongly held by FRANK, STAHL, and their followers have survived the test of experimental inquiry. . . . . In the opinion of the writer, there can be no doubt that recent investigations by means of pure cultures have tended to support the view that possession of mycorhiza is frequently of benefit to the vascular hosts."

The first chapter is devoted to the early period of mycorhizal studies; the second and third to the second period, 1880–1900; the next six chapters to the modern period; the last two chapters to discussion of tuberization and the physiological significance of mycorhiza.—G. K. K. LINK.

Basidiomycetes

A volume by ULBRICH[10] is a revision in the third edition of *Die höheren Pilze* (Basidiomycetes), which is the first volume in the excellent series *Kryptogamen Flora für Anfänger*, inaugurated by the late Professor LINDAU. In harmony with the results of recent research, radical changes were made in the general and in the special parts and keys, so that the presentation is in line with modern concepts and interpretations relative to structure, life histories, and cytology of Basidiomycetes. Although this volume is designed for the use of beginners and amateurs in central Europe, it will be found of considerable use by students of the Basidiomycetes in all parts of the world.—G. K. K. LINK.

[9] RAYNER, M. C., Mycorhiza, an account of non-pathogenic infection by fungi in vascular plants and bryophytes. New Phytol. Reprint no. 15. pp. vi+246. *figs. 64. pls. 7.* London: Wheldon and Wesley, Ltd. 1927.

[10] ULBRICH, E., Die höheren Pilze. Basidiomycetes. Frontispiece portrait of Lindau. xii+497. figs. 38. 14 plates in supplement. Julius Springer. Berlin. 1928.

Paleoecology

A report of the ecological conditions which prevailed during a part of the Permian age in central Germany has been published by WEIGELT.[11] The larger portion of the book is devoted to a description of species, while only a comparatively small part of it deals with the ecological conditions of the period and with other general questions.

The period of the "Kupferschiefers" or copper slate is situated in the lower Zechstein, which, in turn, represents the upper Permian of central Europe. These deposits of copper slate originated in a sea which may have had some similarity to the Black Sea of our age. The shores of this Permian Sea were lined with a vegetation which indicates climatic contrasts with strong insulation and abrupt changes in temperature during which the danger of drought was present.

This vegetation consisted primarily of conifers, Cycadofilicales, ferns, and Equisetales. These plants were characterized by heterophylly, and the author draws interesting conclusions on climatic conditions from this fact. Heterophylly, in our present-day flora, often indicates that the plant organism grows partly in one condition and partly in another. When a plant grows out from the shade into the sunlight, or from moist surroundings into drier ones, we may obtain heterophylly. The conclusions which the author draws from present conditions upon those of the past are very enlightening.—A. C. NOÉ.

Protoplasm

An international periodical on protoplasm[12] was started in July, 1926, devoted to the study of the physical chemistry of protoplasm. The subtitle of the periodical is given in four languages, German, English, French, and Italian. Original articles may be written in any one of these four languages. The purpose of the journal is to unite and to harmonize such sciences as deal with the physical chemistry of protoplasm for the benefit of investigators whose fields are now separated by barriers, but who are all interested in the results of protoplasm research. Among the subjects which the periodical aims to cultivate are colloidal chemistry and microchemistry of protoplasm, protoplasm structure, osmosis, plasmolysis and cytolysis of protoplasm, ultra microscopy as applicable to protoplasm research, the mechanics of cellular and nuclear division; and the pathology of protoplasts. The editors hope that physiologists, cytologists, pathologists, and histologists will be equally interested in this periodical.—A. C. NOÉ.

---

[11] WEIGELT, J., Die Pflanzenreste des mitteldeutschen Kupferschiefers und ihre Einschaltung ins Sediment. Eine paläökologische Studie. pp. iv+395–592. *pls. 35. figs. 14.* Berlin: Gebrüder Borntraeger. 1928.

[12] Protoplasma, edited by J. SPEK (Heidelberg) and J. WEBER (Graz) in collaboration with R. CHAMBERS (New York) and W. SEIFRIZ (Philadelphia). Berlin: Gebrüder Borntraeger.

Plant geography

The first series of monographs in plant geography[13] published by FISCHER in Jena has been completed. Each number contains the monographic treatment of several species, genera, or even families of plants, and is well supplied with maps. There are usually ten maps to a number, and 10–16 pages of text. Each plant type is treated by a competent author, and the names of the best German plant taxonomists are represented on the list. The publishers call this series an "Archiv," in which maps of the natural distribution of all plant groups will be collected and explained. The main object is to give diagrammatic maps of the distribution of recent and fossil plants in order to supply a basis for floristic analysis, plant distribution, and the evolution of floral areas. Also, certain phylogenetic problems of theoretic taxonomy are to be explained by these maps. It is not to be expected that the series can be completed in a few years, and therefore the character of this publication is bound to be that of a periodical.— A. C. NOÉ.

Anatomy of monocotyledons

A very important anatomical study of the monocotyledons[14] is more than half completed. It is planned to comprise seven volumes, with a total of about 800 pages. Four of these volumes have already appeared. The first one treated Pandanales, Helobiae, Triuridales; the second, the Glumiflorae; the third, the Principes, Synanthae, Spathiflorae; and the fourth one, which has recently appeared, the Farinosae. The fifth volume will discuss Liliiflorae; the sixth one Scitamineae and Microspermeae; and the seventh will contain a discussion of the monocotyledons in general and an index for the entire publication. The individual volumes are being published at intervals of about six months, and the entire publication is intended to be an exhaustive anatomical reference book, with special consideration of the taxonomy of the monocotyledons.— A. C. NOÉ.

Reference book of plant anatomy

The largest existing reference book on plant anatomy is being edited by K. LINSBAUER,[15] of Graz, Austria. A number of European scholars from different countries are cooperating in the publication of this monumental work, which is intended to comprise our present-day knowledge of plant tissues. It is still impossible to say how many volumes will appear and when the entire publica-

[13] Die Pflanzenareale, edited by E. HANNIG and H. WINKLER, in collaboration with L. DIELS and G. SAMUELSSON. 1st series. nos. 1–8. 4to. Jena: Gustav Fischer. 1926–1928.

[14] SOLEREDER, H., and MEYER, F. J., Systematische Anatomie der Monokotyledonen. Vol. IV. Farinosae. 8vo. pp. 176. *figs. 65*. Berlin: Gebrüder Borntraeger. 1929.

[15] Handbuch der Pflanzenanatomie, edited by K. LINSBAUER. 8vo. Berlin: Gebrüder Borntraeger. 1922.

tion will be completed. Vols. I–X have appeared to date, either in part or in full, and they show how thoroughly the subject of plant anatomy is to be covered. The entire literature is considered, to judge from the bibliography quoted in this volume. It is to be hoped that this very important publication will soon be completed.—A. C. Noé.

NOTES FOR STUDENTS

New stem type from Devonian rocks of Australia.—A new Devonian stem has been found in the Burdekin beds, Burdekin basin, Queensland, and sent to SEWARD of Cambridge University, England, for examination. He turned it over to HARRIS for examination and description.[16] It is of special interest that the stem is from the Middle Devonian, and therefore throws light upon the divertment of the stele. Every contribution to our knowledge of Devonian plant structures is helpful, and it is fortunate that in the past few years a number of Devonian plant structures have been described.

The most interesting comparison of *Schizopodium davidi* is with *Asteroxylon*, which was first described by KIDSTON and LANG, and later by KRÄUSEL and WEYLAND. The main differences between the two genera can be summarized in the following statement: the new genus has no leaves, but has on the other hand a sort of secondary wood. Also the stele in *Schizopodium* has the shape of the xylem, which is commonly a star, while in *Asteroxylon* the stele is round, even when the xylem is star-shaped.—A. C. Noé.

An interesting plant hybrid.—The raising of hybrids between sugar-cane and cholam (*Andropogon sorghum*) has just been successfully accomplished by Mr. RAO BAHADUR T. S. VENKATRAMAN,[17] Imperial Cane Breeding Station, Coimbatore. As an intergeneric hybrid (by no means too common in the vegetable kingdom) this should prove of considerable scientific interest. The three such hybrids of possible economic value known at present are the "maize-teosinte," the "wheat-rye," and the "cabbage-radish" hybrids. The F_1 generation of sugar-cane being noted for diversity among the progeny, and sugar-cane, as a crop, being propagated vegetatively, it is not impossible that important economic results might come from a "cane-cholam" hybrid.

---

[16] HARRIS, T. M., *Schizopodium davidi* gen. et. sp. nov.: a new type of stem from the Devonian rocks of Australia. Phil Trans. Royal Soc. London. Series B **217**:395–410. *pls. 91–93.* London. 1929.

[17] Madras Agric. Jour. **17**:no. 11. p. 359. 1929.